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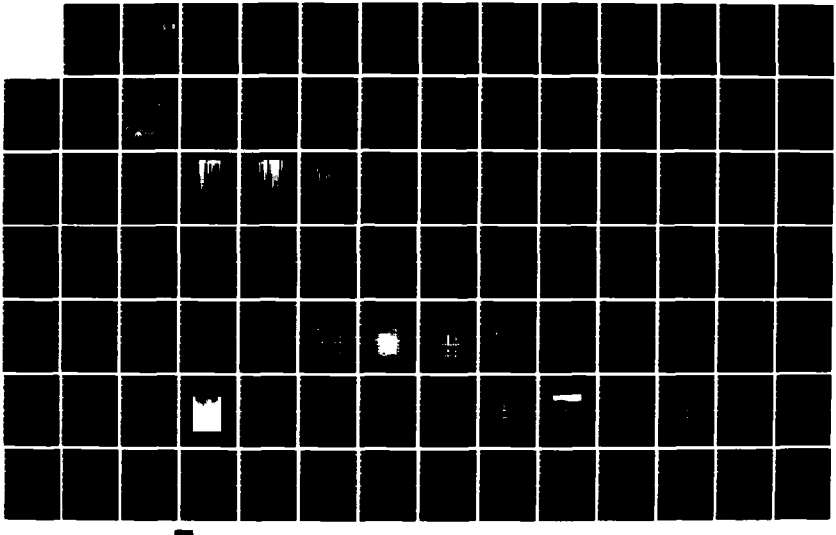

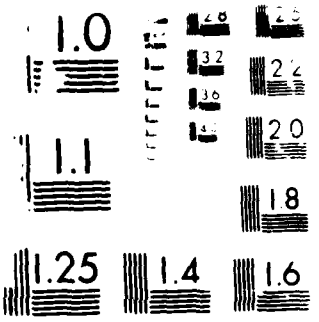
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WORLD REFERENCE CENTER FOR ARBOVIRUSES

ANNUAL PROGRESS REPORT

Robert E. Shope, M.D.

January 1986

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U.S. Army Research and Development Command, Fort Detrick,
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virus identification	rapid diagnosis
dengue	orbivirus

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Viruses were identified in the families Bunyaviridae, Reoviridae, and Flaviviridae. A new orbivirus, Essaouira, was identified from ticks taken on an island off the coast of Morocco. Inkoo virus of the California serogroup was recognized for the first time in Sweden. A new virus in the Tete serogroup was identified from a mynah bird of India. Another new virus, Precarious Point was shown to be in the Uukuniemi serogroup. This virus was from Australia. Chagres virus of the Phlebovirus genus was identified for

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the first time in Colombia, and 2 more new phleboviruses were characterized from Brazil. Toscana, also of the Phlebovirus genus, was isolated from the CSF of a Swedish tourist in Portugal. This is the first time this virus has been implicated in human disease. Naples and Sicilian strains were isolated from sera of febrile persons in Cyprus, and Naples from a child in Egypt.

A revision of the Reoviridae, genus Orbivirus was undertaken incorporating polyacrylamide gel electrophoretic RNA patterns and RNA-RNA hybridization. Strains from Indonesia exhibited 9-, 10-, and 12-segment patterns. The 12-segmented patterns differed from those known from Colorado tick fever isolates.

Diagnosis of disease. Cases of human and equine encephalitis in Connecticut during 1985 were not diagnosed with available arbovirus antigens and antibodies.

Serologic surveys. Serosurvey of residents of a farm in Zambia where Rift Valley fever (RVF) had occurred were positive and the positivity correlated with proximity to farm animals. RVF antibodies were found for the first time in sera of sheep in Mali. ELISA was used to survey for antibody in hospital-associated patients in Uganda. Antibodies to Ilesha, Bunyamwera, RVF, Crimean-Congo hemorrhagic fever (CCHF), and chikungunya were identified.

Pathogenesis of arbovirus infections. Sequential infection of hamsters with phleboviruses Arumowot and Chagres did not lead to immune enhancement. The animals responded with depressed viremia and antibody response after the second infection.

Development of new techniques. RNA-RNA blot hybridization was developed for dsRNA viruses. Individual genes were probed to show that sequence divergence occurs among closely related strains. The technique was used to compare viruses in the Palyam, Corriparta, and Colorado tick fever serogroups. Markedly different patterns of gene evolution and geographic distribution were detected.

SDS treatment of orbivirus antigens significantly increased the sensitivity of the ELISA. The ELISA was also developed for orbivirus antigen detection from ticks.

A dengue-2 anti-idiotypic antibody was produced in rabbits. Experiments are continuing to characterize the antibody and to confirm its specificity for dengue-2 antibody.

Collection of low-passage arbovirus reference strains. Forty more low passage arbovirus strains were added to the YARU collection. These include representative VSV, dengue, EEE, JE, chikungunya, Ross River, and yellow fever isolates.

Distribution of reagents. The reference center distributed 1,050 ampoules of virus stocks, antigens and antisera to laboratories in 21 countries. These represented 200 different viruses.

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SUMMARY

Identification and classification of viruses. Viruses were identified in the families Bunyaviridae, Reoviridae (Orbivirus), and Flaviviridae. A new orbivirus, Essaouira, was identified from ticks taken on an island off the coast of Morocco. Inkoo virus of the California serogroup was recognized for the first time in Sweden. A new virus in the Tete serogroup was identified from a mynah bird of India. Another new virus, Precarious Point was shown to be in the Uukuniemi serogroup. This virus was from Australia. Chagres virus of the Phlebovirus genus was identified for the first time in Colombia, and 2 more new phleboviruses were characterized from Brazil. Toscana, also of the Phlebovirus genus, was isolated from the CSF of a Swedish tourist in Portugal. This is the first time this virus has been implicated in human disease. Naples and Sicilian strains were isolated from sera of febrile persons in Cyprus, and Naples from a child in Egypt.

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FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

BODY OF REPORT

I. IDENTIFICATION AND CLASSIFICATION OF VIRUSES

REOVIRIDAE, Orbivirus

Identification of viruses from Canada, Australia, Finland, France, and California, USA.

CanAr 231, CanAr 232, CanAr 233, CanAr 234, CanAr 245 (A.J. Main, J. Oprandy, T. Schwan). These 5 strains were isolated from individual adult female Ixodes uriae collected on Great Island, Newfoundland, during July 1985. The strains were collected in a study to detect changes in the RNA profiles over time in Kemerovo group viruses in an island ecosystem. Complement-fixation (CF) tests (Table 1) indicated that all five isolates were in the Great Island complex, similar to Great Island and Bauline viruses, two serotypes previously isolated from I. uriae on Great Island during 1971 and 1972. However, migration patterns of RNA segments on polyacrylamide gels were different from previous isolates (Fig. 1). CanAr 245 provided a pattern different from that of the other four isolates which were all identical to each other. The four ticks from which these isoaltes were made were all collected from the same gull chick; five additional ticks from this bird were negative in suckling mice and in BHK cells.

AUS MI-14877 (A.J. Main). Four strains of a Kemerovo group virus were received from Dr. Toby St. George, CSIRO, Brisbane, Australia. They were isolated from Ixodes uriae on Macquaerie Island and tentatively identified as Nugget-like. Because they thawed during shipment, virus was recovered from only one strain. This was a member of the Great Island complex of Kemerovo group viruses by CF (Table 2).

FinV-777, FinV-826, FinV-949 (A.J. Main). Nine strains of Kemerovo group viruses from Ixodes uriae in Norway were received from Dr. Pekka Saikku of Finland. Virus was recovered from only three ampoules; these viruses were identified as members of the Great Island complex by CF tests (Table 2).

Brest/Ar/T222, Brest/Ar/T598 (A.J. Main). These two strains were submitted to YARU for identification by Dr. Claude Chastel of France. They were isolated from Ornithodoros maritimus on two islands off Morocco. Preliminary results demonstrating the relationship of Brest/Ar/T222 with the Chenuda complex of the Kemerovo serogroup were reported in previous Annual Reports. Final results demonstrating that it is a new virus (Essaouira) are shown in Table 3. Work is currently underway with the second virus (Kara Iris) and will be reported later.

Mono Lake isolates (T. Schwan, J. Oprandy, M. Bronson, A.J. Main). Isolates of Kemerovo group viruses recovered from Argas ticks collected at Mono Lake during 1982 and 1984 were identified as Mono Lake virus by ELISA and plaque-reduction neutralization tests (PRNT). RNA profiles were remarkably similar to the prototype strain isolated in 1966. Mono Lake virus grown in Vero cells was examined by transmission electron microscopy. Virus particles were typical for orbiviruses, spherical with differential staining between the inner and outer region, and an average diameter of 70 nm. (Fig. 2).

Table 1. Kemerovo group isolates from individual adult female *I. uriae* collected on Great Island, Newfoundland, Canada, during 1985 compared by complement-fixation tests with earlier isolates.

ANTIGENS	ASCITIC FLUIDS							
	Great I. CanAr 41	Bauline CanAr 14	CanAr 231	CanAr 232	CanAr 233	CanAr 234	CanAr 245	normal
CanAr 41	128/≥128	256/≥128	64/32	64/≥128	64/≥128	64/64	16/16	0
CanAr 14	64/64	128/64	64/32	64/64	64/≥128	64/16	8/8	0
CanAr 231	32/128	16/128		32/32	64/32	8/4	0	0
CanAr 232	128/32	128/32	32/16	128/64				0
CanAr 233	128/128	128/64	64/16		64/32			0
CanAr 234	128/32	128/32	64/16			64/16		0
CanAr 245	128/64	128/32	64/32				0	0
Normal	0	0	0	0	0	0	0	0

* reciprocal of serum titer/reciprocal of antigen titer

0 = <8/<4

Table 2. Identification of unknown viruses from Ixodes uriae collected in Australia (Aus) and Norway (Fin).

ASCITIC FLUIDS

	Fin V-808 Kemerovo group	AusMI-14847 Nugget	Fin V-707 Uukuniemi group
Fin V-808	≥64/≥32	32/8	0
Fin V-777	≥64/≥32	≥64/16	0
Fin V-826	≥64/≥32	≥64/≥32	0
Fin V-949	≥64/≥32	32/16	0
AusMI-14877	≥64/16	≥64/≥32	0
normal	0	0	0

Table 3. Complement-fixation and plaque-reduction neutralization tests comparing Essaouira virus (Brest/Ar/T-222) with other Kemerovo group viruses.

		Essaouira Virus Brest/Ar/T-222			
		antigen		antibody	
VIRUS	STRAIN	CF	NT	CF	NT
Chenuda	EgAr 1152	32/128	10/640	128/128	<10/320
Baku	LEIV 46A	128/256	10/1280	64/128	10/320
Mono Lake	CalAr 861	32/256	<10/640	32/128	<10/320
Huacho	CalAr 883	8/128	10/-	8/128	
Kemerovo	USSR R-10	8/256	<10/-	<8/128	
Tribec	original	<16/64	<10/-	<8/128	
Lipovnik	Cz Lip 91	<8/32		<8/128	
unnamed	FinV-808	<8/128	<10/-	<8/128	
unnamed	FinV-873	<8/256	<10/2560	<8/128	
unnamed	FinV-962	<8/256	<10/-	<8/128	
Tindholmur	DenAr 2	<8/32	<10/20	<8/128	
Mykines	DenAr 12	<8/64	<10/40	<8/128	
Cape Wrath	ScotAr 20	<8/128	<10/80	<8/128	
Great Island	CanAr 41	<8/64	<10/320	<8/128	
Bauline	CanAr 14	<8/128	<10/40	<8/128	
Yaquina Head	RML-15	<8/128	<10/80	<8/128	
Okhotskiy	LEIV 287ka	<8/128	<10/160	<8/128	
Nugget	MI-14847	<8/256	<10/-	<8/128	
Wad Medani	EgAr 492	<8/32	<10/-	<8/128	

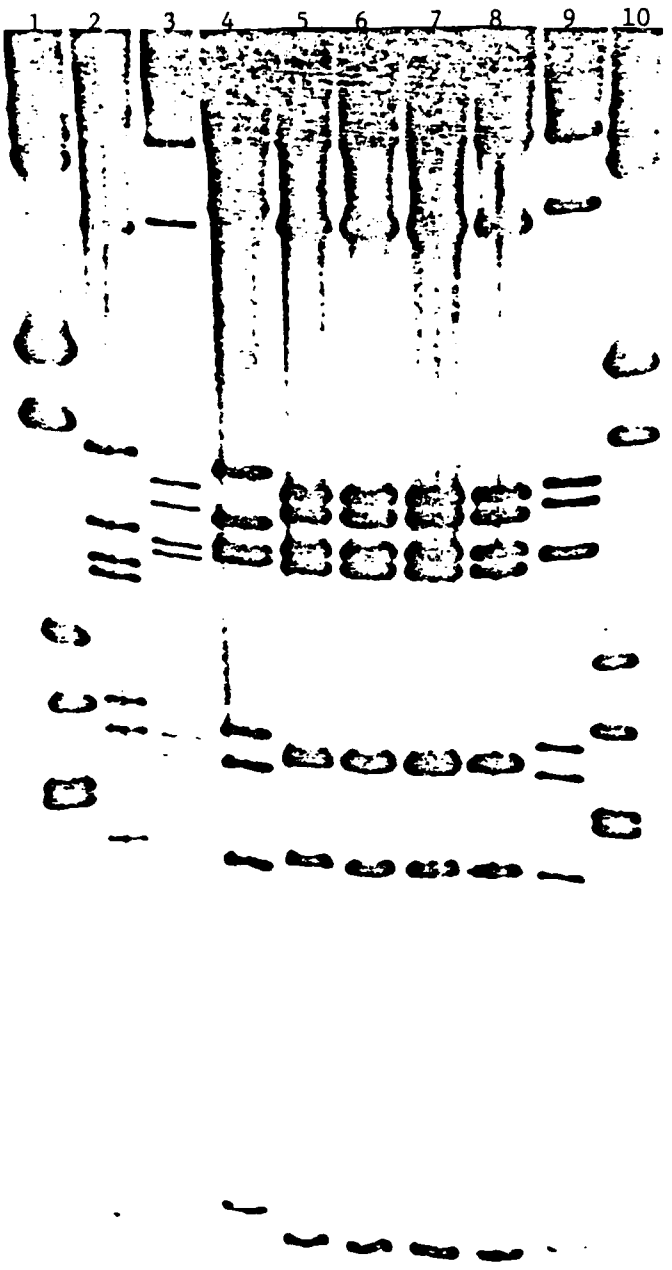


Figure 1 . Autoradiograph of PAGE of 1985 isolates of Kemerovo group viruses from Great Island, Newfoundland, Canada. Lanes 1 to 10 are, respectively: Reo, type 3, Nugget (AusMI 14847), Great Island (CanAr 41), Bauline (CanAr 14), CanAr 231, CanAr 232, CanAr 233, CanAr 234, CanAr 245, Reo, type 3.

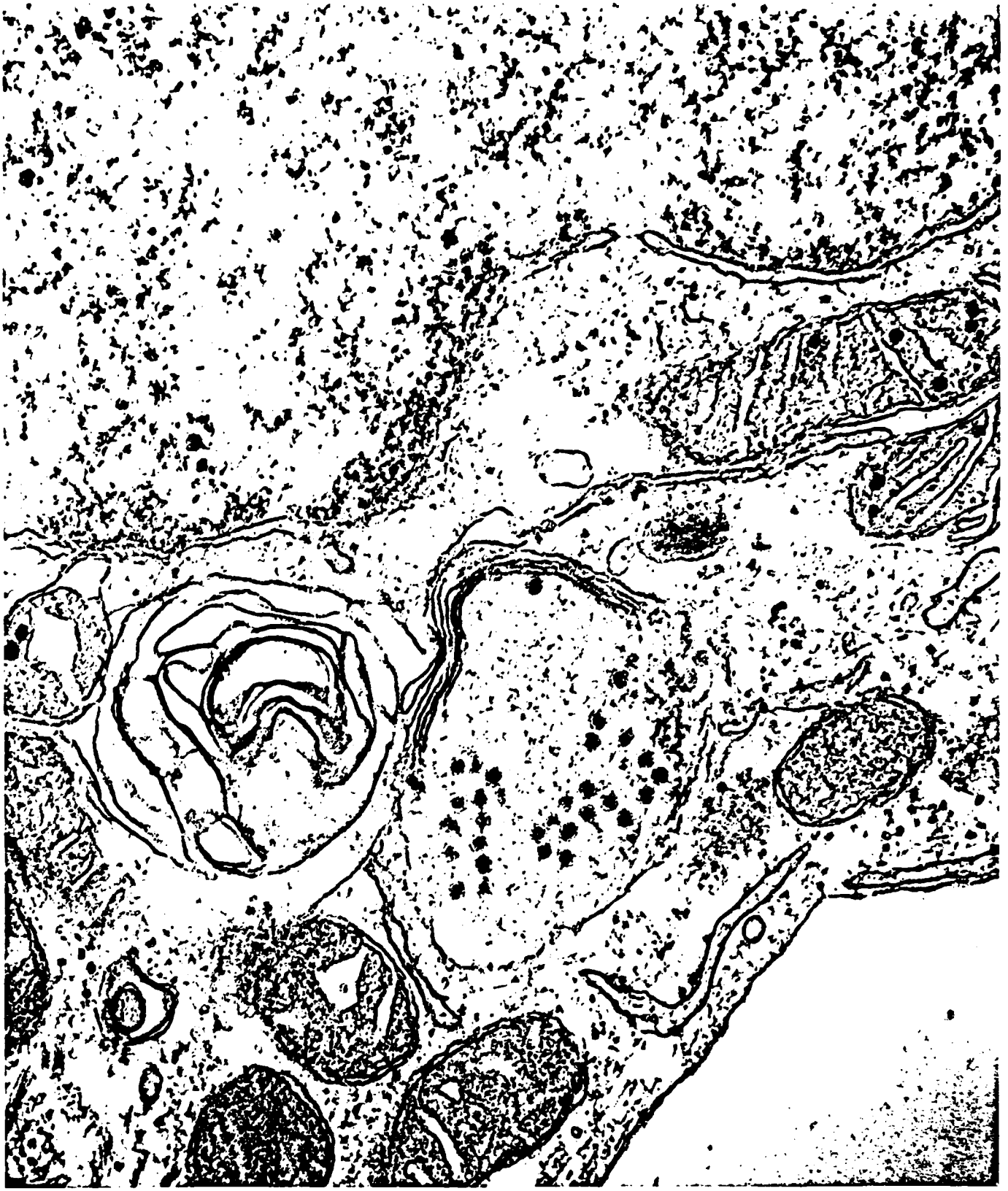


Figure 2 . Electron micrograph of Mono Lake virus in Vero cells.

REOVIRIDAE, Orbivirus

Classification and Taxonomy of Orbiviruses (D.L. Knudson). Table 4 represents a short listing of the current Orbivirus serogroups and isolates based upon serologic data. In concert with the serology, polyacrylamide gel electrophoresis (PAGE) and agarose gel electrophoresis of the genomic dsRNA of these viruses have proven to be useful as initial screening tools to identify presumptive orbiviruses and unidentified viruses. Recently (YARU Annual Report, 1984), RNA-RNA blot hybridization has been utilized to examine the genetic relatedness of orbiviruses within the serogroup (See Section IV.).

Polyacrylamide gel electrophoresis of nucleic acids from viruses isolated in Indonesia (D.L. Knudson and R.B. Tesh). The Indonesian viruses (~200 isolates) which were isolated primarily in mosquito cell lines (YARU Annual Report of 1984) have been examined for the presence of dsRNA. While these data are not shown, the final results are summarized in Table 5. Interestingly, dsRNA PAGE profiles which exhibited 9, 10, and 12 segments of dsRNA were seen. The profiles of the 12 segmented dsRNA viruses were not like those that we have reported for Colorado tick fever virus. Thus, the use of mosquito cell lines as the primary isolation methods has resulted in a large number of novel genotypes. These isolates will be examined by RNA blot hybridization using probes from existing serogroups in an attempt to determine their taxonomic status. As reported last year, one isolate was clearly related to EHD serogroup and this serologic finding has been confirmed by hybridization.

PAGE and Geographic Summaries (D.L. Knudson). Table 6 summarizes the geographic distribution of orbivirus isolates. While it is difficult to assess the significance of the listing, it does indicate the countries which have laboratories actively engaged in virus surveillance programs. Table 7 correlates serogroups with a general dsRNA PAGE profile.

Table 4. ORBIVIRUS SEROGROUPS

AFRICAN HORSESICKNESS SEROGROUP

AHS 1	AHS 6
AHS 2	AHS 7
AHS 3	AHS 8
AHS 4	AHS 9
AHS 5	

BLUETONGUE SEROGROUP

BTV 1 (Biggarsberg)[a]	BTV 13 (160/59 [USA 67-41B])
BTV 2 (22/59)	BTV 14 (87/59)
BTV 3 (Sample B)	BTV 15 (133/60)
BTV 4 (Vaccine-batch 603)	BTV 16 (Pakistan)
BTV 5 (Mossop)	BTV 17 (63-66B [USA 62-45S])
BTV 6 (Strathene)	BTV 18 (South african prototype)
BTV 7 (Utrecht)	BTV 19 (South african prototype)
BTV 8 (Camp)	BTV 20 (Aus CSIRO 19)
BTV 9 (University Farm)	BTV 21 (Aus CSIRO 154)
BTV 10 (Portugal (USA BT8))	BTV 22 (?)
BTV 11 (Nelspoot (USA Station))	BTV 23 (South african ?)
BTV 12 (Byenespoort)	

CHANGUINOLA SEROGROUP

Changuinola (Pan BT 436)	Purus (Be Ar 361064)
Irituia (Be An 28873)	Jari (Be An 385199)
Gurupi (Be Ar 35646)	Saraca (Be Ar 385278)
Ourem (Be Ar 41067)	Monte Dourado (Be An 385401)
Caninde (Be Ar 54342)	Almeirim (Be Ar 389709)
Altamira (Be Ar 264277)	- (Co Ar 2837)
Jamanxi (Be Ar 243090)	

COLORADO TICK FEVER SEROGROUP

CTF (USA Florio)	Eyach (FRG original)

CORRIPARTA SEROGROUP

Acado (Eth Ar 1846-64)	- (Be Ar 263191)
Bambari (Dak Ar B3689)	Jacareacanga (Be Ar 295042)
Corriparta (Aus MRM1)	- (Aus CSIRO 076)
- (Aus CSIRO 109)	- (Aus CSIRO 134)

EPIZOOTIC HEMORRHAGIC DISEASE OF DEER SEROGROUP

EHD 1 (USA New Jersey)	- (SA XBM/67)
EHD 2 (Can Alberta)	- (Aus CSIRO 157)
- (Ib Ar 22619)	- (Aus CSIRO 439)
- (Ib Ar 33853)	- (Aus CSIRO 753)
Ibaraki (Japan 2)	- (Aus DPP 59)
- (Ib Ar 49630)	- (JKT-9133)

EQUINE ENCEPHALOSIS SEROGROUP

7 serotypes

EUBENANGEE SEROGROUP

Eubenangee (Aus IN 1074)	Tilligerry (Aus NB 7080)
Pata (Dak Ar B 1327)	- (Aus CSIRO 20)

Table 4. (continued)

KEMEROVO SEROGROUP

Chobar Gorge (Nep 701700-8)

Great Island complex

Bauline (Can Ar 14)	Nugget (Aus MI-14847)
Cape Wrath (Scot Ar 20)	Okhotskiy (USSR LEIV 70C)
Fin isolates (Fin NorV-808)	Poovoot (USA RML 57493-71)
Great Island (Can Ar 41)	Tindholmur (Den Ar 2)
Kenai (USA 71-1629)	Yaquina Head (USA RML 56297-15)
Mykines (Den Ar 12)	- (UK FT-363)

Chenuda complex

Baku (USSR LEIV 46A)	Mono Lake (Cal Ar 861)
Chenuda (Eg Ar 1170)	Sixgun City (USA RML 52451)
Huacho (Cal Ar 883)	- (Fra Brest Ar T222)

Kemerovo complex

Kemerovo (USSR R 10)	Tribec (Cze original)
Lipovnik (Cze Lip 91)	

Wad Medani complex

Seletar (Mal SM-214)	Wad Medani (Eg Ar 492)

PALYAM SEROGROUP

Abadina (Ib Ar 22388)	Marrakai (Aus CSIRO 82)
Bunyip Creek (Aus CSIRO 58)	Nyabira (792/73)
CSIRO Village (Aus CSIRO 11)	Palyam (I G 5287)
D'Aguilar (Aus B 8112)	Vellore (I 68886)
Kasba (I G 15534)	Petevo (Dak Ar TB 2032)

WALLAL SEROGROUP

Mudjinbarry	- (Aus CSIRO 44)
Wallal (Aus Ch 12048)	

WARREGO SEROGROUP

Mitchell River (Aus MRM 10434)	- (Aus CSIRO 12)
Warrego (Aus Ch 9935)	

UMATILLA SEROGROUP

Umatilla (USA 69-V2161)	Netivot (Isr NT-192)
Llano Seco (USA BFN 3112)	

UNGROUPED

Paroo River (Aus GG 668)	- (USA T-50616)
- (Eg An 1398-61)	Ife (Ib An 57245)
- (Eth Ar 1618)	Japanaut (Aus MK 6357)
- (Eth Ar 3201)	Lebombo (SA Ar 136)
- (Eth Ar 3554)	Orungo (Ug Mp 359)

Ungrouped Mosquito Isolates

9 Segments of dsRNA:

(5-4)

- (JKT-6466)	- (JKT-6612)
- (JKT-6501)	- (JKT-6688)
- (JKT-6502)	- (JKT-6715)
- (JKT-6539)[b]	- (JKT-6732)[b]
- (JKT-6569	- (JKT-7577)
- (JKT-6607)[b]	

10 Segments of dsRNA:

Table 4. (continued)

(2-2-2-2-2)	
- (JKT-6854)[b]	? (JKT-7879)[b]
(2-4-3-1)	
Matsu	- (JKT-8089)
- (JKT-7822)[b]	- (JKT-9891)
(3-3-3-1)	
- (JKT-6512)	- (JKT-9126)[b,c]
- (JKT-8312)[c]	
(3-6-1))	
- (JKT-7781)	- (JKT-9393)
- (JKT-8547)	? (JKT-10087)[b]
12 Segments of dsRNA:	
(6-6)	
- (JKT-6423)	- (JKT-7041)
- (JKT-6969)	
(6-5-1)	
- (JKT-7075)	

[a]Virus designation represents the virus name or - for none and the strain or isolate is listed parenthetically.
[b]Isolate is a mixture with 3 bands present at the top of the gel.
[c]EHD like dsRNA profile.

Table 5. Correlation of DsRNA Profiles of the Indonesian Isolates Made in C6/36 (C) or MOS-61 (M) Dipteran Cells

DsRNA profile (general pattern) Strain	Collection Date	Mosquito Species (pool size)	Indonesian Location
9 Segments of dsRNA: (5-4)			
- JKT-6466	01/05-06/81	Cx. tritaeniorhynchus (50)	Flores
- JKT-6501	01/06-07/81	Cx. tritaeniorhynchus (50)	Flores
- JKT-6502	01/06-07/81	Cx. tritaeniorhynchus (50)	Flores (GP-A)
- JKT-6539[a]	01/07-08/81	Cx. tritaeniorhynchus (50)	Gincu, Flores
- JKT-6569	01/08-09/81	Cx. fuscocephalus (14)	Flores
- JKT-6607[a]	01/10-11/81	Cx. tritaeniorhynchus (50)	Flores
- JKT-6612	01/10-11/81	Cx. tritaeniorhynchus (50)	Flores (BUN, GP-B)
- JKT-6688	01/10/81	Cx. tritaeniorhynchus (18)	Flores (GP-B)
- JKT-6715			
- JKT-6732[a]	02/24-25/81	Cx. tritaeniorhynchus (36)	South Kalimantan
- JKT-7577	02/28/81	Cx. tritaeniorhynchus (50)	South Kalimantan (GP-B)
10 Segments of dsRNA: (2-2-2-2-2)			
- JKT-6854[a]	05/29/81	Cx. quinquefasciatus (4)	Yogyakarta
JKT-7092	06/22/81	Cx. vishnui (33)	Yogyakarta
JKT-7937[a]	06/30/81	Cx. tritaeniorhynchus (50)	Cilacap
JKT-8110	11/21/80	Cx. tritaeniorhynchus (50)	Bali (GP-B)
JKT-8111	11/21/80	Cx. tritaeniorhynchus (50)	Bali (GP-B)
JKT-8130	11/21/80	Cx. vishnui (50)	Bali
JKT-8132[b]	11/21/80	Cx. vushnui (38)	Bali (GP-A)
? JKT-7879[a]	07/25-27/81	Cx. vushnui (51)	Klaten
(2-4-3-1)			
Matsu Group -			
Matsu ?			Taiwan
JKT-7377	02/26/81	Culex spp. (50)	South Kalimantan
JKT-7380	02/26/81	Culex spp. (50)	South Kalimantan (GP-B)
JKT-7398	02/26/81	Culex spp. (50)	South Kalimantan (GP-B)
JKT-7400	02/26/81	Culex spp. (50)	South Kalimantan
JKT-7414	02/26/81	Culex spp. (50)	South Kalimantan (GP-B)
- JKT-7822[a]	02/26-03/10/81	Cx. tritaeniorhynchus (50)	East Kalimantan
- JKT-8089	11/21-22/80	Cx. tritaeniorhynchus (50)	Bali
JKT-8106[a]	11/21/80	Cx. tritaeniorhynchus (50)	Bali
JKT-8118[a]	11/21/80	Cx. fuscocephalus (50)	Bali (GP-B)
JKT-8126	11/21/80	Cx. vishnui (50)	Bali
- JKT-9891	10/14/81	Cx. vishnui (50)	Cilacap
(3-3-3-1)			
- JKT-6512	01/06/81	An. subpictus (50)	Flores
EHD like -			
- JKT-8312	12/08-09/80	An. vagus (68)	Bali
- JKT-9126[a]	03/26/81	An. vagus (50)	Bali
? JKT-9127[a]	03/26/81	An. vagus (50)	Bali (GP-B)
? JKT-9128[a]	03/26/81	An. vagus (50)	Bali
? JKT-10757[d]	11/28/81	An. vagus (?)	Cilacap
- JKT-9133[a,c]	03/26/81	An. vagus (50)	Bali

Table 5. (continued)

(3-6-1)				
-	JKT-7781	02/26-03/10/81	Cx. tritaeniorhynchus (50)	East Kalimantan (GP-B)
-	JKT-8547	01/19-20/81	An. annuloris (27)	Bali
	JKT-8650	01/21/81	An. vagus (50)	Bali
-	JKT-9393	03/30/81	Cx. vishnui (50)	Bali
	JKT-10064[a]	10/21/81	An. subpictus (50)	Cilacap (GET,CHK)
	JKT-10081[a]	10/29-30/81	Cx. vishnui (50)	Cilacap
	JKT-10314	09/02/82	Cx. tritaeniorhynchus (50)	Sumatra
	JKT-10321	09/02-03/82	Ma. uniformis (16)	Sumatra
	JKT-10371[a]	01/11/82	Cx. tritaeniorhynchus (40)	Yogyakarta
	JKT-10394[a]	01/26/82	Cx. tritaeniorhynchus (4)	Yogyakarta
?	JKT-10087[a]	10/29-30/81	Ma. uniformis 2)	Cilacap
	JKT-10274[a]	09/01-02/82	Ma. uniformi 34)	Sumatra
	JKT-10298[a]	09/02/82	Cx. tritaeniorhynchus (50)	Sumatra
	JKT-10304[a]	09/01-02/82	Cx. gelidus (36)	Sumatra
	JKT-10370[a]	01/11/82	Cx. tritaeniorhynchus (50)	Yogyakarta
	JKT-10395[a]	01/26/82	Cx. vishnui (40)	Yogyakarta
12 Segments of dsRNA:				
(6-6)				
-	JKT-6423	?/?/80	Cx. vishnui (36)	Klaten
	JKT-6425	?/?/80	An. vagus (70)	Klaten
	JKT-6429	?/?/80	An. & Cx. spp. (50)	Klaten (GP-B)
	JKT-6434[e]	?/?/80	Culex spp. (100)	Klaten
-	JKT-6969	06/09/81	An. vagus (53)	Yogyakarta
	JKT-6993[e]	06/14-15/81	Cx. vishnui (40)	Yogyakarta
	JKT-10577	11/04/81	Cx. fuscocephalus (50)	Cilacap
-	JKT-7041	06/22/81	An. vagus (50)	Yogyakarta
	JKT-7042	06/22/81	An. aconitus (14)	Yogyakarta
	JKT-7043	06/22/81	An. subpictus (14)	Yogyakarta
(6-5-1)				
-	JKT-7075	07/08/81	Cx. fuscocephalus (29)	Yogyakarta

Dash in the first column denotes a unique dsRNA profile and no dash implies that the dsRNA profile is indistinquishable from the above isolate. The heading of (5-4) denotes the general pattern of dsRNA segments from the largest to the smallest segments. The parenthetic comment in the location column notes that the material is reactive with hyperimmune mouse ascitic fluids to other arboviruses, such as, GP-A = alphaviruses, GP-B = flaviviruses, and other noted following the recognized abbreviation for the virus.

[a]Isolate is a mixture with 3 bands present at the top of the gel.

[b]Kills mice.

[c]Cross-reactive with EHD in CF tests.

[d]Isolate is a mixture with 2 bands present at the top of the gel.

[e]Isolate is a mixture with 5 bands present in the middle of the gel.

Table 6. Geographic Distribution of Orbivirus Serotypes

Serogroup	Number of Serotypes	Geographic Distribution of Serotypes						Vector(s)
		Africa	Europe	Asia	Australia	N. America	Central & South America	
African horsesickness	9	+	+	+				Culicoides
Bluetongue	23	+	+	+	3	5	+	Culicoides
Changuinola	>12						12	Lutzomyia mosquitoes
Colorado Tick Fever	2		1			1		ticks
Corriparta	6	2			2		2	mosquitoes
Epizootic Hemorrhagic Disease of Deer	12	4		1	5	2		Culicoides
Equine Encephalosis	7	7						Culicoides
Eubanangee	4	1			3			mosquitoes
Kemerovo:	23	2	8	4	1	8		ticks
Chenuda	7	1	2	1		3		ticks
Great Island	11		4	1	1	5		ticks
Kemerovo	3		2	1				ticks
Wad Medani	2	1		1				ticks
Palyam	10	3		3	4			Culicoides mosquitoes ticks
Wallal	2				2			Culicoides mosquitoes
Warrego	2				2			Culicoides mosquitoes
Umatilla	3?			1		2		mosquitoes
Ungrouped:	?	7			2	1		mosquitoes
Mosquito isolates -	?			28				none ?
Totals:	>111	>26	>9	>37	22	19	>14	

Table 7. Correlation of DsRNA Profiles with Orbivirus Serogroups

Number of dsRNA Segments	General dsRNA Profile	Serogroup	Number of Serotypes	Vector(s)
9	5-4	Ungrouped mosquito isolates	?	?
10	2-2-2-2-2	Ungrouped mosquito isolates	?	?
	2-4-3-1	Corriparta	6	mosquitoes
		Kemerovo:	23	ticks
		Chenuda	7	ticks
		Great Island	11	ticks
		Kemerovo	3	ticks
		Wad Medani	2	ticks
		Ungrouped mosquito isolates	?	?
	3-3-3-1	African horsesickness	9	Culicoides
		Bluetongue	23	Culicoides
		Epizootic Hemorrhagic Disease of Deer	12	Culicoides
		Eubanangee	4	mosquitoes
		Wallal	2	Culicoides mosquitoes
		Warrego	2	Culicoides mosquitoes
		Umatilla	3?	mosquitoes
		Ungrouped mosquito isolates	?	?
	3-3-4	Changuinola	>12	Lutzomyia mosquitoes
		Palyam	10	Culicoides mosquitoes ticks
		Ungrouped:		
		Orungo	?	mosquitoes
	3-6-1	Ungrouped mosquito isolates	?	?
10?	?	Equine Encephalosis	7	Culicoides
10?	?	Ungrouped:	?	mosquitoes none
12	4-6-1-1	Colorado Tick Fever	2	ticks
	6-5-1	Ungrouped mosquito isolates	?	?
	6-6	Ungrouped mosquito isolates	?	?
			>111	

Characterization of the genome RNA of Kemerovo group viruses (J. Oprandy, T. Schwan, A.J. Main). The genomes of Kemerovo group viruses consist of 10 dsRNA segments which may encode 11 to 13 polypeptides. The functions of these proteins and their respective genome segments are not known. Genome coding assigments and viral assembly may differ from that of other Orbivirus serogroups. The significance of the electrophoretic patterns of RNA of Kemerovo group viruses analyzed to date is still undetermined, but indicates heterogeneity within the genus Orbivirus. Genetic variability is a common occurrence in Reoviridae.

Great Island (GI), Bauline (BAU), and Mono Lake (ML) viruses are three Kemerovo group orbiviruses associated with seabirds and ticks. GI and BAU are transmitted among alcids (Atlantic puffins (Fratercula arctica) and Larids (herring gulls (Larus argentatus), greater black-backed gulls (Larus marinus), and black-legged kittiwakes (Rissa tridactyla) by the 2- (male) or 3- (female) host tick, Ixodes uriae (Ixodidae), on Great Island in the Witless Bay Seabird Sanctuary, Newfoundland, Canada. ML is transmitted among California gulls (L. californicus) by the multi-host tick, Argas n.sp. near cooleyi (Argasidae) on several islands in Mono Lake, California, USA. The genomic segments of GI, BAU, and ML were compared on polyacrylamide gel electrophoresis (Table 8).

Strains of GI and BAU isolated during 1971 and 1972 on Great Island, Newfoundland, Canada were highly variable in the migration patterns of the dsRNA segments by PAGE analysis. GI and BAU both had 10 genome segments in a 2-4-3-1 arrangement that differed from other members of the Great Island complex (Fig. 3). No genome segment co-migrated in all of the samples tested, although segment 1 differed only slightly. Estimated molecular weights of Great Island complex virus genomes averaged 11.99 Megadaltons (Md) with a range of 1.37 Md (Table 9). GI genomes averaged 11.69 Md with a range of 0.29 Md (S.D. = 0.107) (Fig. 5). BAU genomes averaged 11.25 Md with a range of 0.17 Md (S.D. - 0.075). GI PAGE profiles bore little resemblance to other members of the Great Island complex with the exception of BAU. Prototype strains CanAr 41 (GI) and CanAr 14 (BAU) are closest in total genomic molecular weight of all Great Island complex viruses, differing by about 1%.

Conversely, 20 strains of ML isolated over an 18 year period at Mono Lake, California, USA were much less variable. Members of the Chenuda complex also had 10 genome segments in a 2-4-3-1 arrangement (Fig. 4). PAGE profiles of the 20 ML isolates were remarkably similar in their distribution of genome segments. The prototype (CalAr 861) and 1982 isolate (CalAr 1416) differed only slightly in the migration of segments 8 and 10. Segments 1,2,7, 8 and 10 co-migrated in all 1984 samples tested. Estimated total molecular weight of Chenuda complex virus genomes averaged 12.1 Md with a range of 0.44 (Table 9). ML genomes averaged 12.16 Md with a range of 0.11 Md (S.D. = 0.036) (Fig. 5).

Failure of in vitro passage of ML through 8 serial passages in BHK cells to alter genomic migration patterns suggests that variation is the result of processes other than random mutation or infidelity in copying genomic RNA during viral replication. At present these processes are unknown, but a comparison of the ecology of these viruses may help to elucidate them. The relative simplicity of these two ecosystems (Witless Bay and Mono Lake) makes them ideal for study.

The existence of multiple genotypes of closely related viruses in one ecosystem deserves further examination. This has been reported with other orbiviruses (bluetongue, African horse sickness, Changuinola) but in more complicated ecosystems. Several hypotheses have been suggested:

1) that each genotype attained its identity in separate areas before being brought together on Great Island. The recovery of Mykines virus in adjacent colonies on the Faeroe Island, might support this hypothesis. At least one Kemerovo group virus (Kemerovo) was recovered from the blood of a migrating bird. Certainly, local and transoceanic flights of seabirds from Greenland and Europe to Newfoundland are well documented. These include puffins from Scotland where Cape Wrath and Shaints viruses were reported and kittiwakes from the USSR, The Faeroe Islands, and Norway where Okhotskiy, Mykines and Tindholmur, and unnamed members of this complex were isolated. There are many additional areas where the existence of Kemerovo group viruses might be assumed but have yet to be surveyed. However, the RNA profiles of GI and BAU resemble each other more closely than they resemble other members of the Great Island complex.

2) that each genotype developed in separate demes on Great Island. The I. uriae from which GI and BAU were isolated were collected from a variety of habitats on Great Island. Most of the strains were from ticks collected in puffin burrows or from herring gull chicks. Both of these species occur together in the same habitats. More isolates from a variety of habitats will be necessary to examine this question. In addition to the puffins, kittiwakes, herring gulls, and black-backed gulls already mentioned, Leach's petrels (Oceanodroma leucorhoa), common murres (Uria aalge), razorbills (Alca torda), and black guillemots (Cepphus grylle) occur on Great Island. However, all do not serve equally well as host of the tick (and presumably the viruses). The two most abundant species are the puffins and the petrels, each with about 200,000 adult birds on the Island at the time GI and BAU were isolated. It has been reported that 18.4% of the adult puffins in Witless Bay during 1972 and 1973 were infested with an average of 15.6 I. uriae; few ticks were found on puffin chicks. No ticks were observed on more than 500 adult petrels examined during 1971 and 1972 (Main et al., 1976a). However, GI and/or BAU neutralizing antibody was detected in 6% of the petrels tested indicating that this species is at least occasionally infested. This compares with 54% of the seropositive puffins. Whether this is a habitat difference or a physiological or immunological difference associated with host suitability is not known. Both species nest in burrows but in different habitats on different parts of Great Island. Gull populations on Great Island were estimated at 3,000 adult herring gulls and 80 adult greater black-backed gulls. Chicks of both species were heavily infested. Kittiwake chicks were also infested although this species may not be a suitable host for tick development. In one study, 53.6% of the adult common murres were infested with an average of 4.6 ticks; chicks were rarely infested.

3) the genes of orbiviruses are occasionally reassorted giving rise to new variants. On Great Island, where there is a limited period (April-August) of intense transmission, the opportunity for dual infections either in the tick or the avian host exists. The likelihood of this phenomenon occurring in the tick is increased if transovarial transmission occurs. While there is no evidence for this with orbiviruses, Sakhalin virus (Bunyaviridae: Nairovirus) was recovered from the progeny of naturally infected I. uriae in the USSR. Examination of the RNA profiles from additional isolates on Great Island for shared segments is needed.

4) the antigenic composition is constantly changing in response to extrinsic pressures such as immunity levels in the vertebrate host populations. On Great Island, the minimum infection rate for GI dropped from 1:103 in 1971 to

1:617 in 1972; the rate for BAU increased from 1:598 to 1:120 in the same period. The RNA profiles of GI and BAU more closely resemble each other than they resemble other members of the Great Island complex. In addition, strain CanAr 32 (GI) is similar but not identical to two strains of BAU, namely CanAr 128 and CanAr 133. The strains of GI and BAU were separated by neutralization tests; however, only one-way crosses were observed with CanAr 128 and CanAr 133 and the prototype strain of BAU (i.e. both strains were neutralized by hyperimmune ascitic fluids prepared against CanAr 14, but not CanAr 41; neither CanAr 14 nor CanAr 41 were neutralized by CanAr 128 or CanAr 133). Neutralization between prototype GI (CanAr 41) and CanAr 32, while two-way, was only partial. While this may suggest genetic drift, the evidence is only circumstantial. Examination of additional strains, particularly from subsequent years, is necessary. Neutralizing antibody rates in adult puffins (2 years old) on Great Island in 1972 were the same for the two viruses (GI: 37.0%) with 20% of the sera neutralizing both viruses. Two new RNA patterns were observed in isolates from Great Island during 1985.

The rate and direction of dissemination of Kemerovo group viruses by seabirds is unknown. PAGE analysis may be useful in determining this. The wide distribution of Kemerovo, Uukuniemi (Bunyaviridae: Uukuvirus), Sakhalin, and B (Flaviviridae: Flavivirus) group viruses in the northern and southern hemispheres coinciding with the distribution of I. uriae and of Kemerovo and Hughes (Bunyaviridae: Nairovirus) group viruses associated with ticks of the Ornithodoros capensis complex in the tropics and subtropics indicates that the dispersal of viruses does occur. The most likely means of dissemination is either viremic seabirds or virus-infected ticks transported by seabirds. Although transoceanic flights of young (3 year old) kittiwakes and puffins and annual north-south migrations, including flights of certain species between the Arctic and Antarctic occur regularly, these journeys take place after the peak feeding period (late June - early July) of I. uriae in Witless Bay. During the nesting season when I. uriae is feeding, interaction among alcids from distant colonies is uncommon. The integrity of the subspecific races supports this hypothesis. Therefore, the dissemination of virus-infected ticks by migrating birds or by viremic birds is probably a rare occurrence, although the duration of viremia in the avian hosts is unknown. The unique genotypes of the Kemerovo group viruses in each seabird colony separated by more than a few kilometers suggest that the movement of viruses is not a common occurrence or, if it is, that the rate of genetic change in these viruses is rapid.

One recent mechanism for the dispersal of these viruses involves attempts to reestablish certain avian species in areas where breeding colonies existed in the past. One successful attempt involved moving young puffin chicks from Great Island to Egg Rock, Maine. It is not known if Kemerovo group viruses were introduced with the birds but if strains are isolated in the future, PAGE analysis may be useful in determining their origin.

The ecology of Mono Lake differs in many aspects from that of Great Island. These differences may relate to degree of variation seen in the dsRNA of the viruses. California gulls (L. californicus) are the only important vertebrate host for the ticks and virus. Caspian terns (Hydroprogne caspia) have nested in parts of Mono Lake in recent years but the numbers (10-38) are small compared with the 40,00-50,000 adult California gulls breeding there. Between 1953 and 1973 the gull populations increased at an average of 10.6% year. This is a rate of increase that cannot be explained by the production of young at the colony.

Therefore, gulls must have immigrated from other colonies to contribute to the expanding breeding population at Mono Lake. This growth was probably part of a larger expansion throughout western North America. Between 1930 and 1980 the U.S. population increased annually at about 2% and the number of known colonies increased from 15 to 80. A recent genetic analysis of California gulls from Mono Lake and Great Salt Lake showed no genetic variation between these colonies suggesting further that gene flow between these populations may be considerable. Except at Mono Lake, ticks and arboviruses have not yet been associated with California gulls in any other breeding colonies or in their winter grounds along the west coast of the New World from British Columbia to Guatemala. No ticks were found in California gull colonies at Great Salt Lake or South San Francisco Bay, California. The coastal distribution of gulls during the winter does overlap in some regions with the known ranges of other ticks (I. uriae, I. signatus, O. capensis complex) and viruses (Yaquina Head, Farallon, Tyuleniy, Tillamook, Oceanside) associated with breeding colonies of other seabirds. However, the potential for infestation by these ticks and infections with these viruses is unlikely during the winter months and, to date, there is no evidence of this having occurred. Therefore the potential for dual infections in gulls with related Kemerovo group orbiviruses at Mono Lake is much less likely compared with birds at Great Island.

While Mono Lake is a relatively simple ecosystem, clearly it has a history of instability that has potentially influenced the interaction between virus, ticks, and gulls. ML virus was first isolated in 1966. During the 18 years that have elapsed, the number of adult gulls breeding on the islands has increased at least 4-fold (estimated 12,000 to 50,000) with birds immigrating from other colonies an important component of this increase. Also during this period new islets have either formed or increased in size, were joined to the adjacent mainland by landbridges, and then shrunk to become islets again, or submerged as the level of the lake increased or decreased. These physical changes influenced the exposure of gulls to ticks and virus. It is assumed that gulls are required to maintain this virus and are responsible for infecting new generations of ticks, although if transovarial transmission does occur, a vertebrate host may not be necessary. Gull chicks are heavily infested with all stages of Argas ticks with infestations of larvae being intense during late June and early July. California gulls mature in 3 or 4 years and will not nest during this period. The immune status of birds, to either ticks or to viruses, returning to breed is not known. We can speculate, however, that the prevalence of ML antibody in gulls should be lower than the prevalence of GI and BAU antibodies in puffins on Great Island. Reasons for this would be the rapidly growing population of gulls at Mono Lake being comprised, in part, of birds from other colonies presumed to be tick and virus free and the erratic nature of gull nesting from year to year due to changes in nesting habitats.

GI and BAU exhibit a greater degree of genetic variation than ML. The mechanisms for this variation are unknown. Two possible sources are the vertebrate host and its immunological state (herd immunity) and the invertebrate host, in which there may be a greater opportunity for dual infection and genetic reassortment, especially if transovarial transmission is a factor. The role of these two hosts needs further study. During 1985, an attempt was made to collect new isolates of another Chenuda complex virus associated with seabirds and Argasid ticks. However virus was not recovered from 234 Ornithodoros amblus (220 nymphs, 10 adult males, and 5 adult females) from Huacho, Peru. Ticks were tested in suckling mice and in Vero cells.

Table 8. Virus strains used in RNA profile studies.

VIRUS	STRAIN	PASSAGE HISTORY *	YEAR	SOURCE	REGION
Great Island complex:					
Cape Wrath	ScotAr 20	sm 3/BHK 1	1973	I. uriae	Scotland
Nugget	AusMI-14847	sm 6/BHK 3	1975	"	Australia
Great Island	CanAr 41	sm 4/BHK 2	1971	"	Canada
"	CanAr 32	sm 4/BHK 1	"	"	"
"	CanAr 40	"	"	"	"
"	CanAr 42	sm 5/BHK 1	"	"	"
"	CanAr 45	"	"	"	"
Bauline	CanAr 14	sm 3/BHK 2	1971	"	"
"	CanAr 63	sm 2/BHK 1	1972	"	"
"	CanAr 128	"	"	"	"
"	CanAr 133	"	"	"	"
"	CanAr 174	sm 3/BHK 1	"	"	"
Chenuda complex:					
Chenuda	EgAr 1152	sm 18/BHK 1	1966	A. hermanni	Egypt
Huacho	CalAr 883	sm 7/BHK 1	1972	O. amblus	Peru
Sixgun City	RML 52451	V2/sm1/V1/sm1/BHK1	1972	A. cooleyi	USA
Mono Lake	CalAr 861	sm 7/V2/BHK1	1966	Argas sp. n.	"
"	CalAr 1416	sm2/BHK2/V2/BHK1	1982	"	"
"	CalAr T7	V1/BHK1	1984	"	"
"	CalAr S11	"	"	"	"
"	CalAr S18	"	"	"	"
"	CalAr S57	"	"	"	"
"	CarAr M32	"	"	"	"
"	CalAr M33	"	"	"	"
"	CalAr M34	"	"	"	"
"	CalAr N23	"	"	"	"
"	CalAr N69	"	"	"	"

* sm = suckling mice, V = Vero cells, BHK = Baby Hamster Kidney cells

Table 9. Calculated molecular weights of viral dsRNA segments.

	Molecular weight (Megadaltons)								
	Great Island Complex				Chenuda Complex				
Segment	GI	CW	BAU	NUG	CHEN	HUA	SC	ML66	ML82
1	2.48	2.51	2.48	2.56	2.47	2.46	2.46	2.50	2.50
2	2.18	2.20	2.28	2.14	2.20	2.17	2.17	2.20	2.20
3	1.27	1.80	1.33	1.36	1.35	1.30	1.39	1.35	1.35
4	1.27	1.58	1.22	1.22	1.25	1.27	1.30	1.32	1.32
5	1.15	1.30	1.18	1.13	1.21	1.22	1.28	1.25	1.25
6	1.14	1.12	1.13	1.10	1.18	1.14	1.15	1.23	1.23
7	0.66	1.10	0.70	0.79	0.83	0.78	0.78	0.81	0.81
8	0.59	0.61	0.59	0.70	0.72	0.63	0.69	0.63	0.55
9	0.47	0.48	0.46	0.53	0.51	0.41	0.52	0.49	0.49
10	0.36	0.24	0.32	0.21	0.46	0.39	0.47	0.42	0.45
Total	11.57	12.94	11.69	11.74	12.18	11.77	12.21	12.20	12.15

GI = Great Island (CanAr 41)
CW = Cape Wrath (ScotAr 20)
BAU = Bauline (CanAr 14)
NUG = Nugget (AusMI-14847)
CHEN = Chenuda (EgAr 1152)
HUA = Huacho (CalAr 883)
SC = Sixgun City (RML 52451)
ML66 = Mono Lake (CalAr 861)
ML82 = Mono Lake (CalAr 1416)

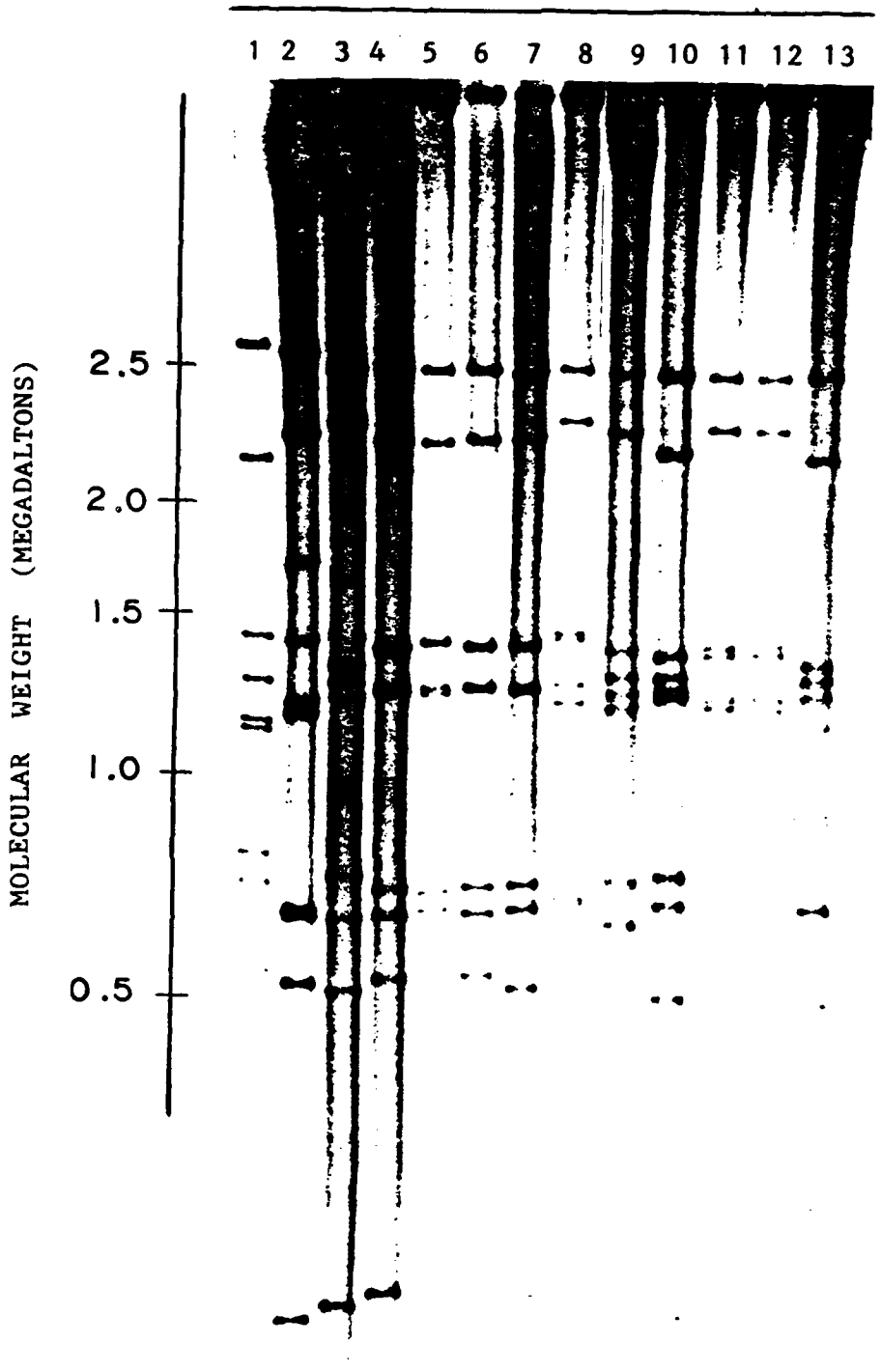


Figure 3. Autoradiograph of PAGE of prototype strains of Great Island complex viruses and strains of Great Island and Bauline viruses. Lanes 1 to 13 are respectively: Nugget (AusMI 14847), Cape Wrath (ScotAr 20), Bauline (CanAr 14), Great Island isolates (CanAr 41, 32, 40, 42, 45), Bauline isolates (CanAr 14, 63, 128, 133, 174).

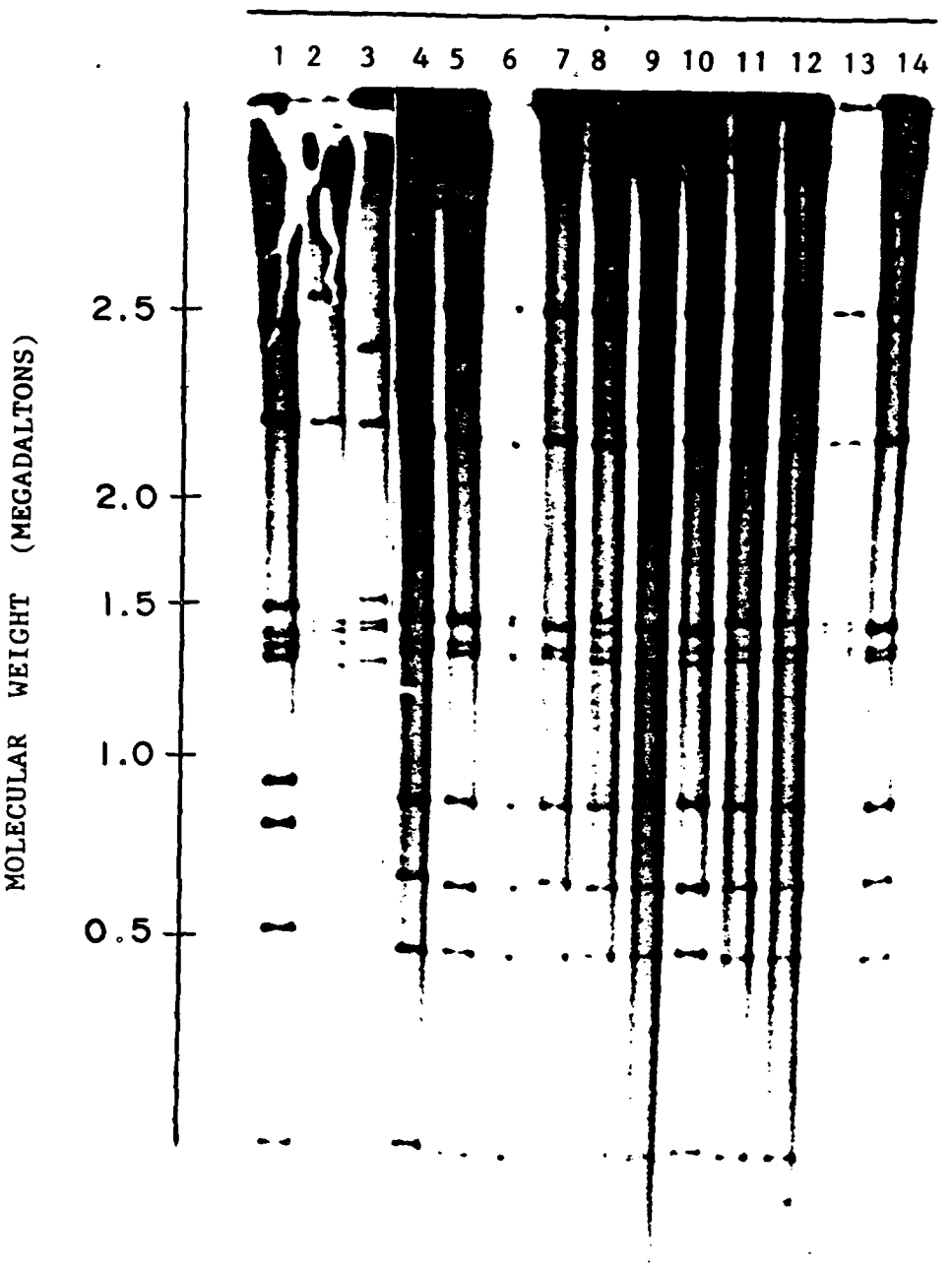


Figure 4. Autoradiograph of PAGE of Mono Lake and other Chenuda complex viruses. Lanes 1 to 14 are respectively: Chenuda (EgAr 1152), Huacho (CalAr 883), Sixgun City (RML 52541), Mono Lake 1966 (CalAr 861), Mono Lake 1982 (CalAr 1416), Mono Lake 1984 isolates (CalAr 17,S11,S18,S57,M32,M33,M34,N23,N69).

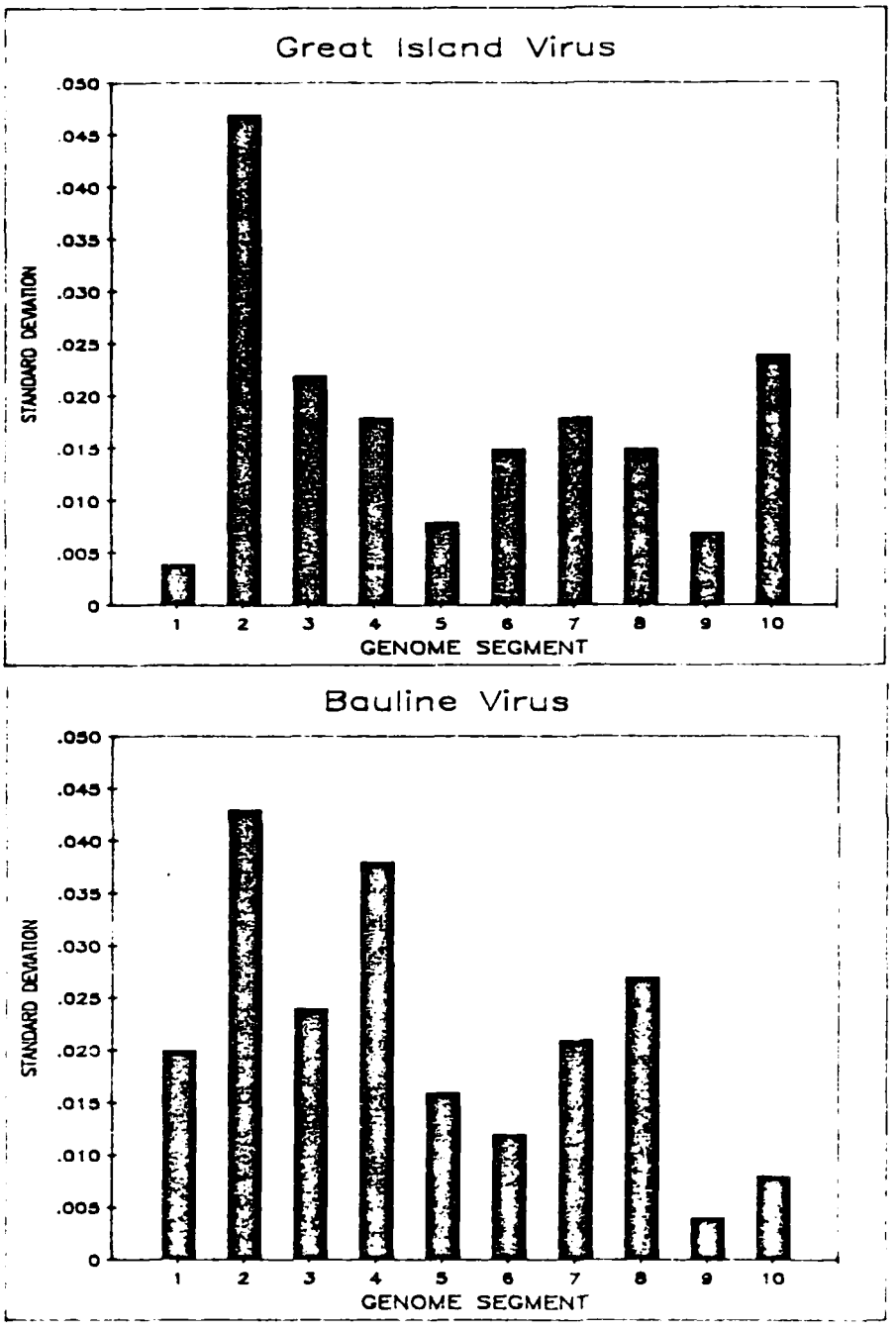


Figure 5. Comparison of variation in the individual genome segments of Great Island, Bauline and Mono Lake virus isolates. Molecular weight was calculated as described for each genome segment of each isolate and the standard deviation determined.

Table 10. Complement-fixation and plaque-reduction neutralization tests comparing RML 66269-34a with other flaviviruses.

	RML 66269-34a			
	ANTIGEN		ANTIBODY	
	CF	NT	CF	NT
Japanese encephalitis	64/64*	80/ -	512/512	
St. Louis encephalitis	128/512	<10/ -	256/1024	
Murray Valley encephalitis	32/64		256/512	
Kunjin	32/64		128/512	
West Nile	32/64		64/512	
US bat salivary gland	128/512		32/1024	
Tyuliney	16/256		32/1024	
Kokobera	8/32		<64/512	
Stratford			<64/512	
Dengue 3	<8/32		64/1024	
Powassan	<8/32		16/1024	
Modoc	8/64		32/1024	
Cowbone Ridge	<8/8		64/1024	
Montana Myotis leucoencephal	<8/≥512		16/1024	

* homologous serum titer/heterologous serum titer

Table 11. Complement-fixation (CF), hemagglutination-inhibition (HI), and plaque-reduction neutralization (PRNT) tests comparing I612045 with other Tete group viruses.

	I612045					
	antigen			ascitic fluid		
	CF	HI	PRNT	CF	HI	PRNT
Tete (SAAn 4511)	128/1024*	80/40	10/320	128/512	10/≥10240	<10/2560
Batama (DakAnB 1292)	128/≥1024	160/-	20/320	128/512	-	<10/2560
Bahig (EgAnB 90)	64/256	20/40	<10/80	256/512	160/≥10240	20/2560
Matruh (EgAn 1047)	32/256	80/-	20/20	<8/512	-	<10/1,280
Tsuruse (Mag 271580)	<8/64	10/320	<10/160	32/512	≥10240/≥10240	160/2560

* heterologous serum titer/homologous serum titer

FLAVIVIRIDAE, Flavivirus

Identification of a flavivirus from ticks collected in Massachusetts (D. Riedel, T. Schwan, A.J. Main). Virus RML 66269-34A was received from Dr. Conrad Yunker when he was at the Rocky Mountain Laboratory in Hamilton, Montana. It was believed to have been isolated from a pool of Dermacentor variabilis collected in Boston, Massachusetts. However, CF tests placed it in the SLE-JE-WN complex of flaviviruses and preliminary PRNT indicates that it is similar or identical to Japanese encephalitis virus (Table 10).

BUNYAVIRIDAE, Bunyavirus

New Indian virus of the Tete serogroup (G. Modi, A.J. Main). The I 612045 virus from the blood of a mynah bird in India was submitted to YARU for identification by Dr. S.N. Ghosh of the National Institute of Virology in Pune. It is a new member of the Tete serogroup by CF, HI, and PRNT (Table 11).

Identification of Inkoo Virus from Sweden (R.B.Tesh and A. Esmark). A virus, designated as 83-161 and isolated from a pool of mosquitoes in central Sweden, was submitted for identification by Dr. B. Niklasson, Department of Virology, The National Bacteriological Laboratory, Stockholm. Virus 83-161 was screened by immunofluorescence and plaque reduction neutralization (PRN) tests against a variety of grouping and specific antisera. In PRN tests with Inkoo virus, it gave the results shown in Table 12. Strain 83-161 reacted reciprocally within a single dilution of Inkoo virus.

On the basis of these results, it was concluded that 83-161 was a strain of Inkoo virus. This isolation represents the first recovery of Inkoo virus from Sweden, although it has been found previously in Norway and Finland. A few cases of febrile illness have been associated with Inkoo virus infection in Finland.

Since Inkoo virus is a member of the California serogroup and a number of viruses in this group have been associated with encephalitis, acute and convalescent sera from 48 Swedish patients with clinical encephalitis were screened by PRN test against Inkoo virus. Seventeen of the 48 patients had Inkoo virus neutralizing antibodies, but the titers were similar in both their acute and convalescent specimens. It was concluded that these individuals had previously been infected with Inkoo virus, but that it was not the cause of their encephalitis.

Table 12

Immunofluorescence reactions of 83-161 with Inkoo virus

	Mouse immune ascitic fluid	
Virus	83-161	Inkoo
83-161	1:5120*	1:640
Inkoo	1:640	1:320

*Highest antibody dilution giving 90% or greater plaque reduction.

BUNYAVIRIDAE, Uukuvirus

Precarious Point, a new Australian virus in the Uukuniemi serogroup (A.J. Main). Strain AusMI-19334 was recently described as a new virus in the genus Bunyavirus (St. George, T., et al., 1985, Am. J. Trop. Med. & Hyg.). Additional CF testing indicates that it is a new member of the Uukuniemi serogroup (Table 13).

Probable new Alaskan virus in the Uukuniemi serogroup (T. Schwan, A.J. Main). Strains RML 105355 and Murre virus were also received from Dr. Yunker. RML 105355 was isolated from a pool of Ixodes uriae and the second strain from the blood of a murre; both are from Alaska. CF tests suggest that they are closely related to each other and probably represent a new member of the Uukuniemi serogroup (Table 13).

BUNYAVIRIDAE, Nairovirus

Identification of a strain of Avalon virus from Canada (A.J. Main, J. Oprandy, T. Schwan). Strain CanAr 252 was isolated from Ixodes uriae collected on Great Island, Newfoundland, during July 1985. CF tests (Table 14) indicate that it is Avalon virus, a Sakhalin group virus originally isolated from Ixodes uriae and a herring gull (Larus argentatus) on Great Island in 1971 and 1972.

BUNYAVIRIDAE (?), genus unknown

Attempts to identify bunya-like viruses from French mammals (A.J. Main). Three strains, Brest/An 219, Brest/an 221, and Brest/an 227, were received from Dr. Claude Chastel of France where he isolated them from various species of small mammals. With much difficulty, two of these strains were finally established in suckling mice at YARU and reagents were prepared. Electron microscopy done in France suggested that they have a bunyavirus-like morphology, although all attempts to demonstrate a relationship by CF with bunyaviruses, nairoviruses, uukuviruses, phleboviruses, and unclassified Bunyaviridae have been negative. The results are shown in Tables 15 and 16; other results have been reported in previous Annual Reports. We are now attempting to develop serodiagnostic reagents for hantaviruses with which to compare these isolates.

BUNYAVIRIDAE, Phlebovirus

Recovery of Chagres virus from Colombian sand flies (R.B.Tesh and J. Boshell) An isolate (CoAr 170391) of Chagres virus was made from phlebotomine sand flies collected in Mariquita, Dept. of Tolima, Colombia in 1985. By plaque reduction neutralization test, this isolate was indistinguishable from the prototype Chagres strain (JW-10) from Panama. This is the first reported isolate of Chagres virus outside of Panama where the virus has been associated with human illness.

Characterization of two new phleboviruses from Brazil and Colombia (R.B.Tesh, J. Boshell and A.P.A. Travassos). Two new phleboviruses (BeAr 407981 and CoAr 170152) were identified during 1985. These agents were originally recovered from phlebotomine sand flies collected in Brazil and Colombia, respectively. Initially, antigens (infected Vero cells) for each of the viruses were examined by indirect fluorescent antibody test (IFAT) against vesicular stomatitis,

Table 13. New isolates compared with other members of the Uukuniemi serogroup by complement-fixation.

ANTIGENS	ASCITIC FLUIDS										
	AusMI-19334	RML-105355	RML-Murre	Fin-V-707	Scot-FT/254	OC RML-38	ZT USSR-LEIV 21c	UUK S-23	GA Argas 27	EgAn 1825-61	MAN Pak-T462
19334	512/≥128*	64/64	256/128	64/32	16/16	0	16/16	64/8	16/16	128/32	0
105355	64/64	≥1024/≥128	512/≥128	16/16	0	0	0	32/8	0	64/16	0
Murre	64/64	≥1024/≥128	512/≥128	64/32	16/16	16/16	32/8	128/8	16/8	256/16	16/4
V-707	32/64	1024/32	128/64	1024/≥128							
FT/254	64/8	64/32	128/8		512/32						
RML-38	32/16	16/32	32/16			256/64					
LEIV 21c	8/8	0	8/8				32/8				
S-23	32/32	256/64	64/32					512/64			
Arg 27	32/32	256/64	256/32						≥1024/≥128		
1825-61	64/64	256/64	128/32							≥1024/≥128	
T 462	8/4	128/16	32/32								≥1024/≥128

* reciprocal of serum titer/reciprocal of antigen titer

0 = <8/<4

Table 14. CanAr 252 compared with other members of the Sakhalin serogroup by complement-fixation.

ANTIGEN	ASCITIC FLUID							
	CanAr 252	Avalon CanAr 173	Clo Mor ScotAr 7	BrestArT261	Sakhalin LEIV 71c	Tillamook RML - 86	Taggert AusMI-14847	Normal
CanAr 252	256/16*	512/8	0	0	0	0	0	0
CanAr 173	256/≥128	>1024/≥128						0
ScotAr 7	8/4		32/32					0
BrestArT261	0			32/64				0
LEIV 71c	16/8				64/32			0
RML-86	8/128					256/≥128		0
MI-14847	32/16						1024/64	0
Normal	0	0	0	0	0	0	0	0

* reciprocal of serum titer/reciprocal of antigen titer

0 = <8/<4

Table 15. List of antibody preparations tested against Brest/An 219 and/or Brest/An 221 antigens in complement-fixation tests during 1985.

POLYVALENT ANTISERA

Group B (8/6/80)
Group B (12/11/66)
Group Phlebotomus fever
Group Phlebotomus fever (Old World)
Group Phlebotomus fever (New World)
Group VSV (Isfahan, Piry, Chandipura)
Group VSV (VSV-NJ, VSV-Ind, Cocal, BeAn 411391)
Group VSV (NJ-IND-COC-BeAn 411391-ISF-PIRY-CHP)
Group Hughes
Group Sakhalin
Group Uukuniemi (3/5/76)
Group Guama
Group Kemerovo
Group Tacaribe

Polyvalent ALM-BEL-CHV-JAP-JOI-MR-WAL-WAR-WON
Polyvalent JA-QRF-KSO-BDA-SIL
Polyvalent JA, etc.
Polyvalent Corriparta, Palyam, etc.
Polyvalent CCHF-HAZ-GAN-DUG-BHA)
Polyvalent Anopheles A, Anopheles B, Turlock, etc.
Polyvalent #3 (KOO-WON-BAK-KET-MAP-TRU-MPK)
Polyvalent #4 (NYM-UUK-GA-THO)
Polyvalent #5 (HUG-SAW-MAT-LS-SOL)
Polyvalent #6 (Marco, Chaco, Timbo, Pacui)
Polyvalent #9 (NAV-TNT-ARU-PCA)
Polyvalent #10 (UPO-DGK-WAN-DHO)
Polyvalent #12 (OKO-OLI-WIT-TAT-BIA)
Polyvalent Rabies, LCM, Herpes, Vaccinia, NDV
Polyvalent Rabies-LCM

SPECIFIC ANTISERA

Brest/An 219	Brest/An 221	MHV (CtAr-159-77)
Aura (BeAr 10315)	EEE (CtAr 70357)	MHV (CtM-1-83f)
KS (EgArT 904)	YOG (DakAn 5634)	MHV (CtAr-232-77)
Hilo (CtAn 114)	COT (SP An 232)	BOC (BT 25)
LC (CSIRO 704)	JKT 2541	TET (Koln 63)
TAM (W-10777)	PIC (An 3739)	AMA (BeAn 70563)
MAC	JUN (XJ)	BMK 1122-70
KOL (DakArB 1094)	BTK (DakA 1077)	I772366-17
LCM (E350)	PAH (Fe 3-52f)	THO
QRF (EgAr 1113)	BBO (DakAnB 1054)	ZGO (DakAnB 1245)
TAC (TRVL 11573)	DHO (cloned)	UPO (C 5581)
AB	JA (LBJ)	RML 64423-8
IK (LEIV 315k)	KET (P6-1361)	CG (701700-8)
O. coriaceus '76	PoTi 461	I 64434
Herpes (MaM 2513b)	Ectromelia (IbAn 34325)	NDV
Whitney's C. gapperi V.	Whitney's Microtus V.	Kwatta
Brest/Ar/T261	Brest/Ar/T439	Ntaya

Table 16. List of antigens tested against Brest/An 219 and Brest/An 221 antibody in complement-fixation tests during 1985.

BT 2365
Catatumbo
EgAn 1047
Bimiti (Tr 8362)
AusCh 9935
Guajara
Punto Toro
Duvenhage
Mucambo (BeAn 8)
BeAn 174214
Manzanilla
AusB 8112
Una (Ar 13136)
DakBA 937
Bebaru
IG 5139
BT 2164
DakArD 14701
IbAn 28946
AusMK 6357
CHIK (IbAn 4824)
I 612629
Kairi
BeAn 123048
Wallal (AusCh 12048)
BeAn 141106
DakAn 1351
Caraparu (BeAn 7981)
DakAr 2053
BT 104
Bakau (MM 2325)
BeAr 177325
AV 137
ISp 68886
Apoi
LeDantec (SH 763)
IbAn 15736
DakAn Y-589
Marituba
Banzi
Highlands J
Belmont (AusR 8659)
BeAn 20076
Langat (TP 21)
FG Ar 564
Una (CoAr 2380)
Israel Turkey encephal
Mouse hepatitis virus
CoAr 2518
I 6701D
Moriche
TP 94
Almpiwar (MRM 4059)
Corriparta
MARU 8179
Nyamanini
Ntaya
Matucare
MARU 8620
IbAn 8341
Tick 39
Oripouche (TR 9760)
BeAn 109303
Nkolbisson (YM 31)
Aura
Kemerovo (R10)
IbAn 20433
SpAn 1098
CoAr 2526
Acara (BeAn 27639)
Dhori (I 611313)
Chagre
Buttonwillow (BFS 5040)
MARU 8563
CoAr 1279
Cedros
AusMK 7937
Mokola
CoAr 1071
Gamboa (MARU 10962)
In 1074
Capim
Calovo (MS 50)
PSC 19
Bukalasa Bat (BP 111)
IG 10658
BeAn 100049
MRM 3029
Entebbe Bat
EgAn 6165
CoAr 50431
Catu (BeH 151)
Hazara (JC 280)
I 47
Bandia
Mt. Elgon Bat (BP 846)
Lumbo
DakA 1077
Gamboa (MARU 11079)
Akabane
Chaco
Whitney's Microtus
Whitney's C. gapperi
CoAr 2837
I 58
TR 9760
M 1146
Murutucu (BeAn 974)
C 505
Brest/Ar/T261
CoAr 2825
CoAr 2088
Gumbo Limbo
EgAn 1825-61
Edge Hill
Kannamangalam
KFD
Pongola (SA Ar 1)
BeAr 41067
Naple Sandfly fever
Kowanyama
BeAr 185559
BT 265
Guama (BeAn 84381)
Minnal (IG 7481)
Batai (IG 20217)
Changuinola
Changuinola
Kaisodi (IG 14132)
Lebombo (SAAr 136)
Bwamba
CoAr 3624
Madrid
CoAr 49888
Flanders
Bangui (Dak HB 754)
Middelburg
Samford (AusB 7974)
Lagos Bat
An 67949
Precarious Point
Murre Virus
Farallon (CalAr 846)
Grand Arbaud
Oriboca
Cambodia
Anopheles A
RML 64423-8
Brest/Ar/T598
Dry Tortugas
Bocas (BT 25)
Bradypus 121
J 134
Jurona
Apuyo
Farallon (CalAr 846)
CoAr 3319
Bushbush
Aruac
Chenuda
Kwatta
Lumbo
LCM
Ieri
Brest/Ar/T439

Changuinola and phlebotomus fever grouping antibodies and against specific phlebovirus immune ascitic fluids (MIAF). Recent experience has shown that the IFAT is as good or better than the hemagglutination-inhibition test for establishing antigenic relationships among phleboviruses. By IFAT, BEAr 407981 antigen reacted with the following immune reagents at a 1:10 screening dilution: Arbia, Arumowot, Belterra, Bujaru, Candiru, Chagres, Icoaraci and Itaporanga. By IFAT, CoAr 170152 antigen reacted with Caimito, Pacui and the phlebotomus fever grouping reagents.

Both viruses were then examined by plaque reduction neutralization (PRN) method against 39 phlebovirus specific immune ascitic fluids. Homologous titers were: BeAr 407981, 1:160; CoAr 170152, 1:1280. By PRN test, BeAr 407981 and CoAr 170152 viruses were not neutralized by any of the following MIAF: Aguacate, Alenquer, Anhanga, Arbia, Arumowot, Belterra, Buenaventura, Bujaru, Cacao, Caimito, Candiru, Corfu, Chagres, Chilibre, Frijoles, Gabek Forest, Gordil, Icoaraci, Itaituba, Itaporanga, Joa, Karimabad, Munguba, Naples, Nique, Oriximina, Pacui, Punta Toro, Rift Valley fever, Rio Grande, Saint Floris, Salehabad, Sicilian, Tehran, Toscona, Turuna or Urucuri.

On the basis of the IFAT and PRN results, we concluded that BeAr 407981 and CoAr 170152 were new serotypes in the phlebotomus fever serogroup (Bunyaviridae: Phlebovirus). The following names are proposed for the new viruses: Ambe for BeAr 407981 and Arboledas for CoAr 170152.

It is noteworthy that we have received several other apparent phleboviruses from Brazil, Colombia and Panama, which to date we have been unable to characterize because they do not produce plaques in Vero cells. These agents all produce viral cytopathic effect in Vero cells; and by IFAT, all react with the phlebotomus fever grouping reagent as well as a number of the phlebovirus-specific MIAF. They also kill newborn mice by intracranial inoculation. However, since they do not produce plaques under agar, we have been unable to do PRN tests to identify them further.

In other biological studies with CoAr 170152 virus, it was shown that sand flies (Lutzomyia gomezi) feeding on a viremic opossum became infected and that virus replication occurred in the insects. Female Lu. gomezi inoculated with CoAr 170152 virus also transmitted the agent to a high percentage (80%) of their F_1 offspring transovarially.

Recovery of Toscana virus from Portugal (R.B.Tesh). A virus, designated ELB, was submitted for study by Dr. Anneka Ehrnst, Central Microbiological Laboratory, Stockholm. It was recovered from the spinal fluid of a Swedish tourist who developed encephalitis shortly after returning from a trip to Portugal. By both complement-fixation and plaque reduction neutralization tests ELB virus was indistinguishable from Toscana virus (Bunyaviridae: Phlebovirus) as shown in Tables 17 and 18.

Table 17

CF test results of ELB virus from human CSF

	Immune ascitic fluid	
Antigen	ELB	Toscana
ELB	≥256/>256*	≥256/>256
Toscana	≥256/>256	≥256/>256

*Reciprocal of highest positive antiserum dilution/highest positive antigen dilution.

Table 18

PRNT results of ELB virus from human CSF

	Immune ascitic fluid	
Virus	ELB	Toscana
ELB	1:5120*	1:2560
Toscana	1:1280	1:1280

*Highest antiserum dilution giving 90% or greater plaque reduction.

Identification of Sicilian- and Naples-related viruses from Swedish U.N. soldiers (R.B.Tesh). During the summer of 1985, two viruses were recovered from the blood of sick Swedish soldiers who were members of the United Nations Peacekeeping Force on Cyprus. These were submitted by Dr. B. Niklasson, Department of Virology, The National Bacteriological Laboratory, Stockholm. By IFAT, one of these agents (R-18) is a member of the Sicilian sandfly fever complex; the other (R-3) is a member of Naples complex. Plaque reduction neutralization tests are now in progress to the specific serotype of each.

Identification of a Naples-like phlebovirus from Egypt (R.B.Tesh). A virus, isolated from a febrile Egyptian child, was submitted by Dr. T.G. Ksiazek, U.S.Naval Medical Research Unit 3, Cairo. Preliminary studies by indirect fluorescent antibody and neutralization tests suggest that this isolate (84-0005) is closely related to Naples sandfly fever virus.

UNCLASSIFIED VIRUSES

Lake Clarendon, an ungrouped virus from Australia (A.J. Main). Lake Clarendon is an ungrouped virus from Argas robertsi from Australia. The lack of relationship by CF to known arboviruses has been shown in previous Annual Reports. During 1985, attempts to demonstrate relationships with the antisera listed in Table 15 and antigens and antisera in Table 19 were negative.

Ungrouped viruses from New York State voles (A.J. Main). Two unrelated viruses, Whitney's Microtus (NY 64-7947) and Whitney's C. gapperi (NY 64-7855), from voles in New York State were tested in CF tests against the reagents listed in Tables 15 and 16. No new relationships were observed.

Ungrouped tick agents from western U.S.A. and Tanzania (A.J. Main). No new relationships were detected with two unrelated, ungrouped tick isolates, Sapphire II and RML 64423-8, from Ornithodoros cooleyi in Colorado and Montana and Argas brumpti from Tanzania, respectively. Antibody preparations to both viruses were tested by CF test with the antigens listed in Table 16.

II. DIAGNOSIS OF DISEASE

Human encephalitis in Connecticut (A.J. Main, T. Schwan). Sera from two cases of encephalitis were received from Dr. Donald Mayo and Ms. Mary Anne Markowski of the Virology Section of the Connecticut Department of Health Services. Sera (V 5002-8274; V 5002-8423) from the first patient (DS), who had a travel history of Connecticut and Brazil, were tested by CF with the antigens listed in Table 20 with negative results. These sera were also negative by ELISA when screened with EEE, Highlands J, SLE, Powassan, Flanders, LaCrosse, Jamestown Canyon, snowshoe hare, Keystone, trivittatus, and Cache Valley antigens. Brain biopsy material from this patient was negative in suckling mice and in Vero cells. The biopsy tissues failed to react in ELISA with "convalescent" sera (7/20/85) from this patient, so it is not surprising that it did not react with EEE, SLE, Aura, Connecticut virus, Jamestown Canyon, Hilo, Embu, Cotia, herpes, or group B mouse ascitic fluids in direct and sandwich (using the non-reactive convalescent serum to coat the plates) ELISA. This may be because there was no antigen present in the biopsy tissues or because of the problems with storage before we received

Table 19. List of antiSERA and antigens tested against Lake Clarendon (CSIRO 704) virus in complement-fixation tests.

Antibody*

Clo Mor (ScotAr 7)	Taggert (MI-14850)	Sakhalin (LEIV 71c)
Avalon (CanAr 173)	Tillamook (RML-860	Tillamook (RML-38)
Brest/Ar/T439	Brest/Ar/T261	Sapphire II
Farallon	Zaliv Terpeniya	Zirqa
Manawa (Pak T 462)	Avalon (CanAr 15)	Soldado
Hughes	Jamestown Canyon	Snowshoe Hare
Trivittatus	Congo (Dak 8194)	Qalyub (EgAr 360)
Grand Arbaud	EgAn 1825-61	FinV-707
Uukuniemi	Punta Salinas (CalAr 888)	Turlock
Anopheles B	Lukuni	Marutuci (BeAn 974)
Ilesha	Tete (SAAn 4511)	Aus 96037
Matruh (EgAn 1047)	Batama (DakAnB 1292)	Bahig (EgB 90)
Keystone	ISS Ph1 18	SFF, Sicilian
Salehabad (I 18)	PAAr 814	BA 365
Umbre (IG 1424)	Tacaiuma (Be 73)	Boracea (SPAr 395)
Bangoran	Pahayokee	Shark River
Bakau (MM 2325)	Precarious Point	Kao Shaun (EgArT 904)
Nyamanini		Lake Clarendon

Antigens

Soldado	Soldado (SAAr 15908)	Soldado (SenAr-386-77)
Punta Salinas	Zirqa	Hughes
Farallon	Sapphire II	Scot FT/254
Fin V-873	Manawa (PakT 462)	Grand Arbaud
Murre Virus	Sakhalin (LEIV 71c)	Tillamook (USA 86)
Brest/Ar/T261	Brest/Ar/T439	Taggert (AusMI-14850)
Clo Mor (ScotAr 7)	Avalon (CanAn 476)	Avalon (CanAr 15)
Avalon (CanAr 46B)	Avalon (CanAr 173)	Precarious Point
Kao Shaun	Nyamanini	Lake Clarendon

* also see Table

Table 20. Human sera V 5002-8423 and/or V 5002-8274 were screened by complement-fixation against the following antigens.

Alphavirus
- *EEE 3/11/85
- Aura 7/29/68
- *Highlands J (72666) 9/22/76
- Mayaro 5/5/70
- Mucambo 5/23/83
- Pixuna (BeAr 35645) 9/13/65
- Una 7/22/68
- VEE 12/28/78
- WEE 10/15/84

Flavivirus
- Bussuquara 8/12/68
- Dengue 3 12/9/75
- Ilheus 3/13/85
- *SLE (BeAr 23379) 5/1/84
- yellow fever (F2015) 9/4/67
- *Powassan 7/1/75
- Tyuleniy 12/27/75
- Cowbone Ridge 8/22/68

Bunyavirus
- Anopheles A 12/12/73
- Anopheles B 5/30/67
- Tacaiumi (BeAn 73) 7/21/66
- *Cache Valley (61D240) 6/21/67
- Maguari (BeAr 7272) 8/23/68
- Oropouche (BeAn 19991) 3/19/85
- Turlock 1/9/75
- Apeu 6/2/67
- Caraparu (BeAn 7981) 5/7/75
- Itaqui 1/30/68
- Madrid 4/25/69
- Oriboca 1/24/68
- *Jamestown Canyon 11/22/66
- *Keystone (Ar 1274-76) 1/23/78
- *LaCrosse 12/6/66
- Melao (Tr9375) 1/9/75
- Serro do Navio (BeAn 103645) 5/29/75
- *snowshoe hare 10/4/66
- *trivittatus
- Patois 5/10/67
- Capim 8/28/68
- Gamboa (MARU 10962)
- Catu (H151) 3/9/66
- Guama (An 277) 3/11/66

Phlebovirus
- Candiru (BeH22511) 11/19/65
- Chagres 8/8/68
- Punto Toro 11/7/69

Vesiculovirus
- VSV-Indiana 5/14/75

other Rhabdoviruses
- Hart Park 1/16/75
- Connecticut 8/24/84
- *(Flanders - ELISA only)

Arenaviruses
- Junin (XJ) 12/1/65

* antigens used in ELISA as well as CF tests

the sample. Two day post-inoculation brain suspensions from two of the inoculated mice also failed to react in direct and sandwich ELISA with the same sera and ascitic fluids used with the biopsy material. These two mouse brains also failed to react in CF tests with the convalescent sera or the ascitic fluids listed in Table 21.

An acute serum (5002-978) from a second patient (AH) was negative in CF and/or ELISA with the following antigens: EEE (CF, ELISA), WEE (CF), Highlands J (CF, ELISA), SLE (CF, ELISA), Powassan (CF, ELISA), LaCrosse (CF, ELISA), Jamestown Canyon (CF, ELISA), Keystone (CF, ELISA), trivittatus (CF, ELISA), Cache Valley (CF, ELISA), Flanders (CF), and Connecticut (ELISA) viruses.

Equine encephalitis in Connecticut (A.J. Main, D. Riedel, T. Schwan). Blood and brain from a fatal equine encephalitis case in North Stonington, Connecticut, was received from Ms. Lois Heinz of the Diagnostic Testing Service, Department of Pathobiology, University of Connecticut in Storrs. We were not able to demonstrate antigen in the brain or blood, nor antibody in the serum (Table 22).

III. SEROLOGIC SURVEYS

Serosurvey of biologists (A.J. Main). Serum samples from 10 biologists conducting field studies on Lyme disease were screened by direct and sandwich ELISA for antibody against EEE, Highlands J, WEE, SLE, Powassan, yellow fever, Jamestown Canyon, LaCrosse, snowshoe hare, Keystone, trivittatus, Connecticut, Flanders, rabies, Whitney's Microtus, and Whitney's C. gapperi viruses. All samples were negative at a 1:50 dilution except one which reacted with WEE antigen in both tests.

Rift Valley fever serosurvey of farm residents in Zambia after an outbreak of hemorrhagic fever (G. Tembo, R.E. Shope). During February 1985 a febrile hemorrhagic illness afflicted workers on Frinjilla Farm, Chisamba, Zambia. History obtained from Dr. Robert Swanepoel, Institute of Virology, Sandringham, Republic of South Africa was as follows. There were about 70 black workers and 8 whites on the farm. Half of the black residents became ill early in February and 2 young children died with gastrointestinal signs, compatible with (but not diagnostic of) Rift Valley fever (RVF). Mosquitoes were numerous around the farm during early February.

On Saturday, 16 February four blacks who worked in the dairy became ill. On the same day a servant in the house sickened. On 17 February, one of the white women developed fever. On 18 February, Mr. Woodley, the owner of the farm was sick, and on 19 February Mr. Woodley's son sickened. The son died in Zambia on February 23.

On February 19 and 21 respectively two additional white males sickened and were brought to Johannesburg. RVF virus was isolated from the blood of each patient. Two weeks later, an additional white resident of the farm developed fever but a diagnosis was not made.

Sera were collected by one of us (G.T.) from 80 survivors and were brought to Yale University for ELISA. The results are shown in Table 23. Sera from 48 adults and 32 children were tested. There were 10 positive reactions, all in persons 14 years or older. There was a positive correlation between working in and around the cattle barns and RVF ELISA positivity.

Table 21. Mouse ascitic fluids (MIAF) and human sera (HS) used in complement fixation tests with mouse brains inoculated with brain biopsy material (DS).

Group B		08/06/80	MIAF
Group Phlebotomus fever		02/00/72	MIAF
Group Phlebotomus fever (New World)		02/05/83	MIAF
Group Phlebotomus fever (Old World)		02/05/83	MIAF
Group VSV (VSV-NJ, VSV-Ind, Cocal, BeAn 411391)			MIAF
Group VSV (Isfahan, Piry, Chandipura)			MIAF
Group VSV (NJ, Ind, Coc, BeAn 411391, ISF, Piry, CHP)			MIAF
Group Guama		05/10/66	MIAF
Group Hughes		04/23/74	MIAF
Group Kemerovo		04/00/71	MIAF
Group Tacaribe		05/23/68	MIAF
Group Uukuniemi		03/05/76	MIAF
Group Sakhalin		02/22/74	MIAF
Polyvalent 3 (KOO-WON-BAK-KET-MAP-TRU-MK7532) 5/70			MIAF
Polyvalent 4 (NYM-UUK-GA-THO)		05/00/71	MIAF
Polyvalent 5 (HUG-SAW-MAT-LS-SOL)		05/00/71	MIAF
Polyvalent 6 (Marco, Chaco, Timbo, Pacui)		2/27/81	MIAF
Polyvalent 9 (NAV-TNT-ARU-PCA)		01/00/71	MIAF
Polyvalent 10 (UPO-DGK-WAN-DHO)		09/00/71	MIAF
Polyvalent 12 (OKO-OLI-WIT-TAT-DAK 1569)		11/00/71	MIAF
Polyvalent Johnston Atoll, etc.		06/10/68	MIAF
Polyvalent JA-QRF-KSO-BDA-QYB-SIL		04/09/69	MIAF
Polyvalent Corriparta, Palyam, etc.		07/00/70	MIAF
Polyvalent CCHF-HAZ-GAN-DUG-BHA		03/05/74	MIAF
Polyvalent Australia (ALM-BEL-CHV-JAP-JOI-MR-WAL-WAR-WON) 7/29/76 MIAF			
Polyvalent Rabies-LCM-Herpes-Vaccinia-NDV 3/28/74			MIAF
Polyvalent Rabies-LCM		01/07/69	MIAF
Polyvalent Anopheles A, Anopheles B, Turlock, etc. 6/17/66 MIAF			
Whitney's Microtus	NY 64-7947	11/14/74	MIAF
Whitney's C. gapperi	NY 64-7855	11/22/78	MIAF
Newcastle disease		10/24/69	MIAF
Ectromelia	IbAn 34325	01/02/70	MIAF Poxvirus
Cotia	SP An 232	02/16/71	MIAF Poxvirus
Herpes	MaM 2513b	05/14/71	MIAF Herpesvirus
mouse hepatitis	CtM-1-83f	07/01/83	MIAF Coronavirus
Hilo	CtAn 114	07/00/69	MIAF
Pahayokee	FE3-52F	04/28/67	MIAF Bunyavirus
EEE	CtAr 70357	12/09/74	MIAF Alphavirus
Aura	BeAr 10315	09/15/65	MIAF Alphavirus
SLE	Parton	02/05/74	MIAF Flavivirus
Tacaribe	TRVL 11573	12/11/67	MIAF Arenavirus
LCM	E 350	07/28/75	MIAF Arenavirus
Junin	XJ	11/25/69	MIAF Arenavirus
Tamiami	W-10777	11/27/68	MIAF Arenavirus
Pichinde	An 3739	11/08/67	MIAF Arenavirus
Amapari	BeAn 70563	11/17/67	MIAF Arenavirus
Machupo			MIAF Arenavirus
DS	V5002-8423	07/20/85	HS
AJM		01/03/80	HS

Table 22. ELISA and complement-fixation tests with the blood and brain from a fatal case of equine encephalitis in Connecticut.

	ELISA	sandwich ELISA		complement-fixation		suckling mice	vero cells
	antigen (brain)	antigen (brain)	antibody (serum)	antigen (brain)	antibody (serum)	antigen (brain)	antigen (brain)
EEE	<1:10	<1:10	<1:100	<1:10	<1:2	negative	negative
Higlands J	<1:10	<1:10	<1:100	<1:10	<1:2		
WEE					<1:2		
St. Louis enc.	<1:10	<1:10	<1:100	<1:10	<1:4		
Cache Valley	<1:10	<1:10	<1:100	<1:10	<1:4		
Powassan	<1:10			<1:10	<1:4		
Group B	<1:10			<1:10			
Jamestown Can.	<1:10			<1:10	<1:4		
snowshoe Hare	<1:10			<1:10	<1:4		
Keystone	<1:10			<1:10			
Flanders					<1:4		
Connecticut	<1:10			<1:10			
Rabies				<1:10	<1:4		
Herpes	<1:10			<1:10			
LCM				<1:10			
NDV				<1:10			
vaccinia				<1:10			
85:4009 (acute serum)		<1:10		<1:10			

ELISA of sera of sheep and cattle from Mali (R.E. Shope, R. Yedloutschnig, this project was supported also in part by BARD grant No. US-347-80). ELISA was done with a collection of sheep and cattle sera provided by Dr. Farouk Hamdy of Bamako, Mali. The sera were collected during 1985 in the slaughterhouse and tested at the Plum Island Animal Disease Center, NY. Five per cent of cattle and 8% of sheep were positive. Titers were as high as 1:6400 (Table 23). RVF antibody was described in human residents of Mali in the 1930's, but this is the first evidence to our knowledge of recent RVF infection.

Table 23

Serosurveys for Rift Valley fever:

A. ELISA-positive human sera from Fringilla Farm, Chisamba, Zambia

Sample number	Age	Sex	Titer
105	24	F	1:3200
107	25	F	1:50
201	28	M	1:3200
208	26	F	1:3200
303	20	M	1:6400
306	18	M	1:6400
309	64	M	1:200
404	18	F	1:6400
702	14	F	1:50
1105	43	M	1:50

B. ELISA-positive ovine and bovine sera from Mali:

Ovine 9/115 (8%) positive

Plum Island #	Mali #	Result
322	188	1:100
347	208	1:1600
348	378	1:1600
358	397	1:800
359	145	1:800
386	355	1:200
389	153	1:100
391	143	1:400
399	151	1:100 + (not titered)

Bovine 5/101 (5%) positive

251	44B	1:3200
246	38B	1:6400
268	69B	1:800
278	79B	1:1600
307	33B	1:6400

Survey by ELISA for arbovirus antibodies in Uganda (D. Kiguli, R.E. Shope). Sera from patients entering clinics and hospitals in Uganda were collected for study by ELISA. Initially, 589 sera were screened at the 1:50 dilution with Ilesha, Bunyamwera, Rift Valley fever (RVF), Crimean-Congo hemorrhagic fever (CCHF), and chikungunya antigens. A polyvalent anti-human peroxidase conjugate was used in the screening test. The results are shown in Table 24. Positive reactions were found for all antigens tested. There was focal distribution of some positive reactions; Ilesha reactions were localized primarily in the Uganda Virus Research Institute clinic population, Bunyamwera reactions in Luwero, CCHF reactions in Entebbe Hospital and Luwero, and chikungunya in Entebbe hospital, Luwero, and U.M.P. populations. The significance of this apparent focality is not known.

An attempt was made to see if capture IgM ELISA can be used in selected cases as an indication of the diagnosis at the time the patient visits the clinic or hospital. ELISA-positive sera were tested and several reacted in the IgM capture ELISA, however the results are not yet completely analyzed.

Table 24

ELISA survey of incoming hospital and clinic febrile patients in Uganda

Location	No. of sera	per cent positive				
		Ilesha	Bunyamwera	RVF	CCHF	chikungunya
Uganda Virus Research Inst.	186	7.0	0.5	1.0	2.1	0.5
Nkokonjeni	280	2.1	2.5	1.1	1.1	0.7
Entebbe Hosp.	73	0	2.7	0	6.8	4.1
Luwero	30	0	23.3	0	10.0	3.3
U.M.P.	20	0	0	0	0	10.0

IV. PATHOGENESIS OF ARBOVIRUS INFECTIONS

Sequential infection of hamsters with phleboviruses (R.B. Tesh and M. DuBois). Although the phenomena of "immune enhancement" and "original antigenic sin" have been demonstrated with dengue and several other flaviviruses, little is known about the effect of multiple phlebovirus infections on the vertebrate host. Experiments are now in progress to find out, using 3 phleboviruses (Arumowot, Chagres and Gabek Forest) in adult hamsters. These 3 agents were selected, since they are antigenically related and because they cause detectable viremia in laboratory animals (most phleboviruses do not). Hamsters were sequentially infected by subcutaneous inoculation with various combinations of Arumowot, Chagres and Gabek Forest viruses. The level of viremia and neutralizing antibody titers with each agent were determined by plaque assay. Of special interest are the following: the level of viremia following primary, secondary and tertiary infections; the specificity of the neutralizing antibody response after primary and sequential infections; and the level of protection that immunity to one phlebovirus provides upon subsequent challenge with another. Preliminary results of this study are shown in Tables 25 to 28.

Incomplete results of Experiments 1,2,3 and 4 suggest the following:

1) After subcutaneous inoculation of Gabek Forest virus, adult hamsters develop an overwhelming infection and die rapidly (3-4 days);

2) Hamsters sequentially infected with Arumowot and Chagres viruses develop viremia and monotypic antibody respones after each infection;

3) Immune enhancement did **not** occur with the second phlebovirus infection; in fact, primary infection with Chagres virus seemed to decrease the level of viremia and antibody response during secondary Arumowot infection.

4) Primary infection with Arumowot virus protected hamsters from death when challenged with Gabek Forest virus (results not shown).

Table 25
Experiment 1

Viremia and neutralizing antibody response in hamsters infected sequentially with Chagres and Arumowot viruses*

Animal number	Virus titer in blood post-inoculation** day 1	day 2	day 3	day 4	day 5	Neutralizing antibody titer 3 weeks post-inoculation *** AMT	CHG
A. Primary infection (Chagres)							
2236	0	5.6	5.9	0	0	<10	2560
2237	0	2.2	6.0	5.2	0	<10	160
2238	0	0	5.2	5.4	0	<10	640
2241	0	0	0	2.5	5.6	<10	2560
2263	0	3.6	5.5	5.0	0	-	-
2264	0	0	5.0	0	0	-	-
2265	0	0	4.5	5.3	0	-	-
2267	0	6.0	5.0	0	0	-	-
2270	0	4.4	5.8	0	0	-	-
B. Secondary infection (Arumowot)							
2236	2.3	5.0	6.2	3.5	2.5	-	-
2237	2.0	5.3	6.0	3.3	2.2	40	320
2238	0	4.9	5.4	2.6	2.2	40	320
2241	0	4.4	6.0	0	0	640	2560
2263	0	3.2	4.0	5.6	0	-	-
2264	2.2	4.0	4.7	4.2	0	-	-
2265	2.2	4.8	6.6	5.0	0	-	-
2267	0	5.3	4.8	2.0	2.4	-	-
2270	0	4.7	6.6	4.8	0	-	-

*Hamsters received $10^{3.0}$ PFU of Chagres and $10^{5.4}$ PFU of Arumowot viruses subcutaneously.
**Virus titer in blood expressed as $\log_{10}$ of PFU/ml, 0=<$10^{1.7}$ PFU/ml.
***Reciprocal of highest serum dilution producing ≥90% plaque reduction.

Table 26
Experiment 2

Viremia and neutralizing antibody response in hamsters infected sequentially with Arumowot and Chagres viruses*

Animal number	Virus titer in blood post-inoculation** day 1	day 2	day 3	day 4	day 5	Neutralizing antibody titer 3 weeks post-inoculation *** AMT	CHG
A. Primary infection (Arumowot)							
2229	3.0	6.0	6.7	3.2	0	320	< 10
2231	3.9	5.7	7.0	2.8	0	640	< 10
2234	3.4	6.0	6.2	3.6	0	2560	< 10
2235	5.2	6.2	6.5	1.7	0	160	< 10
2261	2.0	5.5	6.2	2.6	0	-	-
2262	0	5.0	6.3	2.7	0	-	-
B. Secondary infection (Chagres)							
2229	0	4.3	5.6	2.0	0	1280	320
2231	0	4.2	4.0	0	0	1280	320
2234	0	0	0	4.8	3.0	2560	160
2235	0	0	4.7	3.6	0	1280	320
2261	0	0	5.9	5.3	0	-	-
2262	0	0	6.0	5.4	0	-	-

*Hamsters received $10^{5.4}$PFU of Arumowot and $10^{3.0}$ PFU of Chagres viruses subcutaneously.
**Virus titer in blood expressed as $\log_{10}$ of PFU/ml. 0=$<10^{1.7}$ PFU/ml.
***Reciprocal of highest serum dilution producing ≥90% plaque reduction.

Table 27
Experiment 3

Viremia in hamsters following infection with Gabek Forest virus*

	Virus titer in blood post-inoculation**			
Animal number	day 1	day 2	day 3	day 4
2206	0	7.8	9.0	dead
2207	0	7.7	9.0	dead
2252	0	8.3	dead	-
2253	0	8.8	9.2	dead
2254	0	8.0	9.6	dead
2255	0	7.6	dead	-

*Hamsters received $10^{4.6}$ PFU of Gabek Forest virus subcutaneously.
**Virus titer expressed as $\log_{10}$ of PFU/ml. $0=<10^{1.7}$ PFU/ml.

Table 28

Experiment 4

Viremia in hamsters following infection with Arumowot virus*

	Virus titer in blood post-inoculation**				
Animal number	day 1	day 2	day 3	day 4	day 5
2239	3.3	5.7	6.0	3.4	2.2
2219	3.6	4.7	3.9	2.3	0
2256	3.2	4.7	6.3	3.0	0
2257	2.6	6.0	6.8	3.4	0
2259	2.0	4.4	6.3	0	0
2266	0	5.6	6.9	5.6	2.7
2268	0	6.6	6.3	5.3	0

*Hamsters received $10^{5.4}$ PFU of Arumowot virus subcutaneously.
**Virus titer expressed as $\log_{10}$ of PFU/ml. 0= $10^{1.7}$ PFU/ml.

V. DEVELOPMENT OF NEW TECHNIQUES

RNA-RNA BLOT HYBRIDIZATION

Genetic Relatedness of Palyam Serogroup Viruses by RNA-RNA Blot Hybridization (D. K. Bodkin and D. L. Knudson). Cognate genes of members of the Palyam serogroup of orbiviruses have been identified, and their relatedness to the prototype virus was determined previously by blot hybridization of the genome segments of members of the serogroup using Palyam genomic RNA and isolated Palyam RNA segments as probes (Bodkin and Knudson, 1985a). In this study, the genetic relatedness of nine Palyam serogroup isolates was determined by reciprocal blot hybridizations of genomic RNA from each virus to the segments of all members of the group. The number and identity of highly related genes between isolates varied. For example, CSIRO Village and Palyam were related in genes 2 and 6, while Bunyip Creek and Vellore were related in genes 2 and 6. However, CSIRO Village and Bunyip Creek were highly related to D'Aguilar in all genes except 2 and 6, indicating that there may have been genetic reassortment of Palyam serogroup dsRNA segments. Genes 2 and 6 were correlated consistently with serotype-specificity.

Genes 5, 7, and 9 were highly related among all members of the group. The Indian strains, Palyam and Vellore, were highly related in genes 1, 3, and 8; and they exhibited weak homology to genes 1, 3, and 8 of the Australian and African strains. However, one Indian isolate, Kasba, was more closely related to strains from Africa and Australia, than it was to other Indian strains. There was little evidence which indicated that geography was predictive of the genetic relationships of the strains. Thus, immunologic pressure may be the most important factor affecting the Palyam serogroup gene pool.

Genetic Relatedness of the Indian Strains. In the discussion of all hybridization data, segments are consistently referred to according to their cognates in Palyam virus (Bodkin and Knudson, 1985a). Since the sixth segment from the top of the D'Aguilar, Bunyip Creek, Kasba, and Petevo dsRNA polyacrylamide gel profiles cross-hybridized to gene 5 of Palyam (Bodkin and Knudson, 1985a); it will be referred to as gene 5. Likewise, the fifth segment from the top of the gel in these isolates will be referred to as gene 6.

Based on the intensity of the hybridization signals, it was revealed that of the three Indian strains, Palyam and Vellore were more closely related than were Palyam and Kasba (Bodkin and Knudson, 1985a). In order to confirm that the intensities of the hybridization signals were consistent in reciprocal reactions, Vellore and Kasba genomic RNAs were used as probes (Figs. 6a and b). Palyam genes 1, 3, 5, and 7 to 9 exhibited strong signals when hybridized to radiolabelled Vellore genomic RNA (Fig. 6a, lane PAL), while genes 4, 5, 7, 9 and 10 of Palyam exhibited strong signals when hybridized to Kasba genomic RNA (Fig. 6b, lane PAL). These results were consistent with the previous experiments (Bodkin and Knudson, 1985a). Of the 30 hybrid molecules which can be compared reciprocally in the Palyam, Vellore, and Kasba blots (Bodkin and Knudson, 1985a; Fig. 6a, lanes PAL and KAS; Fig. 6b, lanes PAL and VEL), only genes 1 and 3 of Kasba and Vellore were not consistent in reciprocal reactions. Kasba genes 1 and 3 exhibited dark signals when Vellore genomic RNA was the probe (Fig. 6b, lane KAS), but Vellore genes 1 and 3 exhibited light signals when Kasba genomic RNA was the probe (Fig. 6a, lane VEL).

Abadina was the only strain whose genes 2 and 6 were closely related to

their Kasba cognates (Fig. 6b, lane ABA). Marrakai gene 6 exhibited strong homology with its Kasba cognate (Fig. 6b, lane MAR). Since Kasba, Marrakai, and Abadina were included in the same antigenic complex (Knudson et al., 1984), these data were consistent with genes 2 and 6 being correlated with the immunologic specificity of the viruses (Bodkin and Knudson, 1985a). Genes 2 and 6 of Bunyip Creek exhibited homology with their Vellore cognates (Fig. 6a, lane BC) as would be expected from the cross-reactivity of these viruses in neutralization tests (Knudson et al., 1984; Bodkin and Knudson, 1985a).

It has been demonstrated that viruses isolated within the same geographic area may be closely related genetically. For example, Gorman et al. (1981) found that there was little sequence homology between Australian and South African bluetongue strains, whereas South African strains have been shown to exhibit considerably higher levels of sequence homology (Huismans and Howell, 1973). In the present study, geographic boundaries appeared to be of little importance in determining the genetic relatedness of the isolates. For example, Kasba was isolated in Vellore, India in 1957, while Abadina was isolated in Africa in 1967, and Marrakai was isolated in Australia in 1975 (Knudson et al., 1984). Yet, Kasba exhibited a higher degree of relatedness to these two isolates than it exhibited with Palyam, which was isolated in Vellore, India in 1956 (Knudson et al., 1984). In certain instances, strains isolated in distant geographic locales may be more closely related than some of those isolated within close proximity of one another.

Genetic Relatedness of the Australian Strains. Previous biochemical and biological investigations have indicated that Bunyip Creek, D'Aguilar, and CSIRO Village might represent a gene pool of closely related strains. For example, several combinations of two of these three have been isolated from individual cattle (Cybinski and St. George, 1982). Knudson et al. (1984) observed that genes 1 and 3 to 10 of these three strains exhibited identical electrophoretic mobilities in polyacrylamide gels.

D'Aguilar genomic RNA was hybridized to the genome segments of Palyam serogroup viruses which had been transferred from polyacrylamide gels to membranes (Figs. 7a and b). D'Aguilar gene 2 did not hybridize to the heterologous samples. Gene 6 of D'Aguilar (which is the fifth segment from the top of the gel) did not cross-hybridize to its cognates in 7 of the 8 isolates, while the cross-hybridization signal of the remaining isolate, Palyam virus, was light when compared to the other Palyam genes. In contrast, Palyam gene 6 exhibited a high degree of relatedness to its cognate in CSIRO Village (Bodkin and Knudson, 1985a). Therefore, D'Aguilar gene 6 appeared to share minimal sequence homology with its cognates in the other members of the serogroup.

CSIRO Village and Bunyip Creek (Fig. 7a, lanes D and F) appeared to be most closely related genetically to D'Aguilar. The consistently strong signals exhibited by the genes of these two isolates contrasted with the relatedness of D'Aguilar to Palyam and Vellore whose genes 1, 3, 4, 6, 8, and 10 exhibited light signals (Fig. 7a, lanes B and J). Among the remaining isolates genes 1, 3, and 8 exhibited light signals, with the exception of Kasba genes 1 and 3 (Fig. 7b, lane D).

The samples were hybridized to pCp labelled genomic RNA from Bunyip Creek (Fig. 8a). Although the exposure was light when compared to the other experiments, differences in the intensities of hybridization signals were discernible. Genes 1, 3 to 5, and 7 to 10 of Abadina, CSIRO Village, and

D'Aguilar exhibited strong signals (Fig. 8a, lanes ABA, CV, BC; gene 5 in D'Aguilar is the sixth segment from the top of the gel). Kasba genes 1, 3, 7, 9, and 10 exhibited strong signals (Fig. 8a, lane KAS). The genes of Palyam, Vellore, Petevo, and Marrakai appeared to be more distantly related to their end-labelled cognates in Bunyip Creek than did those of the other strains. In the hybridization experiment using pCp labelled genomic RNA from CSIRO Village as the probe, the relative intensity of the signals again indicated a high degree of relatedness among Bunyip Creek, CSIRO Village and D'Aguilar (data not shown).

It is well recognized that segments with identical electrophoretic mobilities may not have identical sequence composition (Walker et al., 1980). However, the hybridization analysis supported the electrophoretic data which suggested high levels of relatedness among eight genes of these three strains. Given that CSIRO Village and Bunyip Creek exhibit Palyam- and Vellore- "like" genes 2 and 6, the relatedness of CSIRO Village, Bunyip Creek, and D'Aguilar genes 1, 3 to 5, and 7 to 10, may be evidence for reassortment of genome segments. Taken together with the surveys demonstrating that the strains co-circulate in nature (Cybinski and St. George, 1982), the hybridization analysis may indicate that these three Australian isolates represent a common gene pool through which functionally equivalent, but genetically distinct segments 2 and 6 circulate.

Hybridization of Marrakai genomic RNA to the genome segments of Palyam serogroup viruses is depicted in Figure 8b. Marrakai gene 2 did not cross-hybridize to the heterologous samples. Kasba and Abadina genes 1, and 3 to 10 (Fig. 8b, lanes KAS and ABA) exhibited a high degree of relatedness to their Marrakai cognates. Genes 1, 3 to 5, 7, and 9 of Bunyip Creek and D'Aguilar exhibited strong signals (Fig. 8b, lanes BC and DAG). Genes 4, 5, 7, 9, and 10 of Vellore and Palyam exhibited strong signals (Fig. 8b, lanes VEL and PAL), while genes 5, 7, 9, and 10 of Petevo exhibited strong signals (Fig. 8b, lane PET).

The Australian isolate, Marrakai, does not exhibit the same host and geographic range as the three other isolates. It is the only Australian serotype which has not been isolated from cattle in which one of the other strains had been isolated previously (Cybinksi and St. George, 1982). The Marrakai hybridization data supported the conclusion that a gene pool defined by 3 of the 4 Australian isolates exists. The mechanism by which Marrakai remains outside this gene pool has not been elucidated.

Genetic Composition of Palyam Serogroup Viruses. Genomic RNA from Petevo and Abadina was hybridized to membranes containing the segments of Palyam serogroup viruses (data not shown). The results of the blot hybridization experiments (Bodkin and Knudson, 1985a; Figs. 6 to 8; and data not shown) are summarized in Table 29. Table 29 is a more conservative estimate of the extent of sequence variation exhibited by Palyam serogroup viruses than is seen in Figs. 6 to 8, which represent pairwise relationships.

Only segment 2 exhibited more than one unique gene, and segment 6 exhibited the greatest number of variants. These data were consistent with the previous suggestion that gene 2 may encode the neutralization antigen of the Palyam serogroup (Bodkin and Knudson, 1985a). Likewise, gene 6 was also associated more clearly with serotype-specificity than had been demonstrated previously.

It would appear that among Palyam serogroup strains isolated in nature, the evolutionary pressure of the vertebrate immune system is exerted on both genes 2 and 6, such that sequence divergence in gene 2 is accompanied by sequence divergence in gene 6. A similar situation may exist with respect to evolutionary pressures acting on genes 1, 3, and 8 as a unit (Table 29). *In vitro* reassortment studies have not yielded evidence of genetic linkage of dsRNA segments (Mustoe *et al.*, 1978; Ramig *et al.*, 1978; Kahlon *et al.*, 1983; McCance, E. F. and Knudson, D. L., unpublished results). Nor do these data provide evidence of physical linkage of dsRNA segments. However, the data presented here imply that a given subset(s) of the viral genes may be involved in related functions such that they respond to evolutionary pressures interdependently.

The evolutionary relationships of orbiviruses are topics of considerable interest as indicated by a number of studies on this subject (Gorman *et al.*, 1978; Gorman *et al.*, 1981; Huismans and Bremer, 1981; Rao *et al.*, 1983). The results of this study imply that the vertebrate immune system is the major cause of sequence divergence among members of an orbivirus serogroup. Geographic boundaries are less important in determining sequence divergence between strains. However, a comparison of three Australian isolates suggested that locale may be critical for the maintenance of common gene pools which enable viruses to interact genetically. Moreover, a comparison of the amount of sequence variability exhibited by individual genes indicated that certain subsets may maintain similar levels of sequence variability. Continued investigation of the relatedness of orbiviruses by RNA-RNA blot hybridization may provide further insight into the mechanisms by which these viruses respond to evolutionary pressures.

These data are in press in the Journal of General Virology.

Table 29. Conservative Estimates of Genetic Relatedness of Palyam Serogroup Viruses[a]

COGNATE SEGMENT	VIRUSES								
	KAS	PET	MAR	ABA	CV	BC	DAG	VEL	PAL
1	psg[b]	pet	psg	psg	psg	psg	psg	vel-pal[d]	vel-pal
2	KAS-ABA[c]	PET	MAR	KAS-ABA	CV-PAL	BC-VEL	DAG	BC-VEL	CV-PAL
3	psg	pet	psg	psg	psg	psg	psg	vel-pal	vel-pal
4	psg	psg	psg	psg	psg	psg	psg	psg	psg
5	psg	psg	psg	psg	psg	psg	psg	psg	psg
6	kas-aba	pet	kas-aba	kas-aba	cv-pal	bc-vel	dag	bc-vel	cv-pal
7	psg	psg	psg	psg	psg	psg	psg	psg	psg
8	psg	pet	psg	psg	psg	psg	psg	vel-pal	vel-pal
9	psg	psg	psg	psg	psg	psg	psg	psg	psg
10	psg	psg	psg	psg	psg	psg	psg	psg	psg

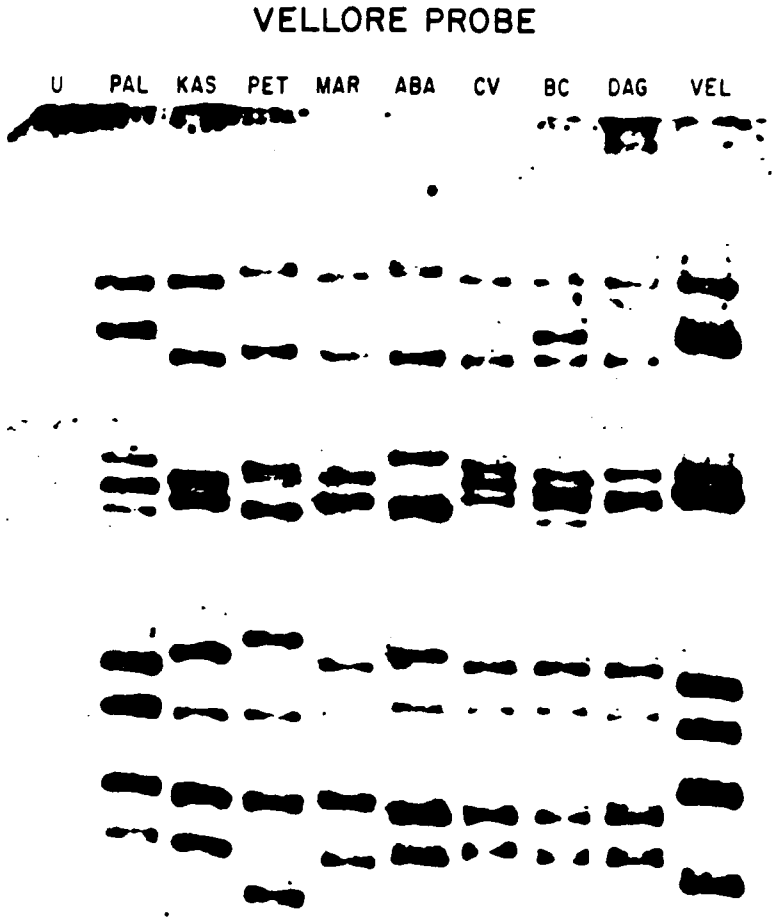
[a]Membranes containing the genome segments of nine Palyam serogroup isolates were hybridized to [5'-^{32}P]-pCp labelled genomic RNA from D'Aguilar (Fig. 7), Kasba (Fig. 6b), Vellore (Fig. 6a), Marraka[illegible] Fig. 8b), Bunyip Creek (Fig. 8a), Palyam (Bodkin and Knudson, 1985a), Abadina (not shown), and Petevo (not shown). The genes were scored as [illegible] Bodkin and Knudson, 1985a).

[b]Segments which exhibited strong hybridiza[illegible] gene designated psg (Palyam serogroup gene). Unique segments [illegible] virus name in capital letters did not cross-hybridize [illegible] same es. Variant segments, designated by the virus name [illegible] etters, hybridized weakly to their cognates when they were used as pr[illegible]es. Moreover, they exhibited weak signals in all reciprocal reactions when hybridized to radiolabelled genomic RNA from the other viruses.

[c]If two viruses exhibited sequence homology in segments which were unique or variant with respect to the remaining viruses, the gene in question is identified by both virus names.

[d]Gene 1 of Kasba exhibited a strong signal when hybridized to Vellore genomic RNA. Genes 3 of Kasba and Abadina exhibited strong signals when hybridized to Vellore genomic RNA. However, Vellore genes 1 and 3 were classified as variant because of their high degree of relatedness to Palyam genes 1 and 3.

Figure 6. Autoradiograms depicting hybridization of Vellore (Fig. 6a) and Kasba (Fig. 6b) genes to their counterparts in the Palyam serogroup viruses. Total genomic dsRNA was end-labelled with [5′-^{32}P]-pCp and hybridized to the genome profiles of nine members of the serogroup. Lanes are uninfected cellular control (U), Palyam (PAL), Kasba (KAS), Petevo (PET), Marrakai (MAR), Abadina (ABA), CSIRO Village (CV), Bunyip Creek (BC), D'Aguilar (DAG), Vellore (VEL). The hybridization in Fig. 6a was performed by removing the Kasba probe from the membrane (Bodkin and Knudson, 1985b) used in Fig. 6b, and rehybridizing the samples to Vellore genomic RNA. The position of segment 2 (which does not cross-hybridize in some isolates) may be determined by examining the control labeled lanes for each virus in Figure 7.



KASBA PROBE

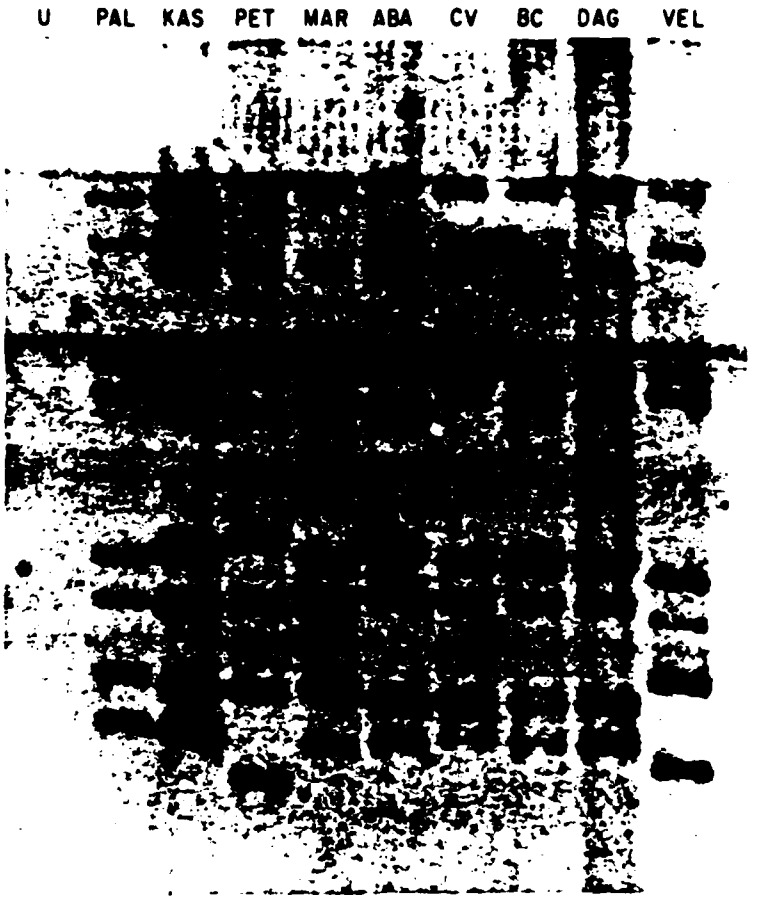
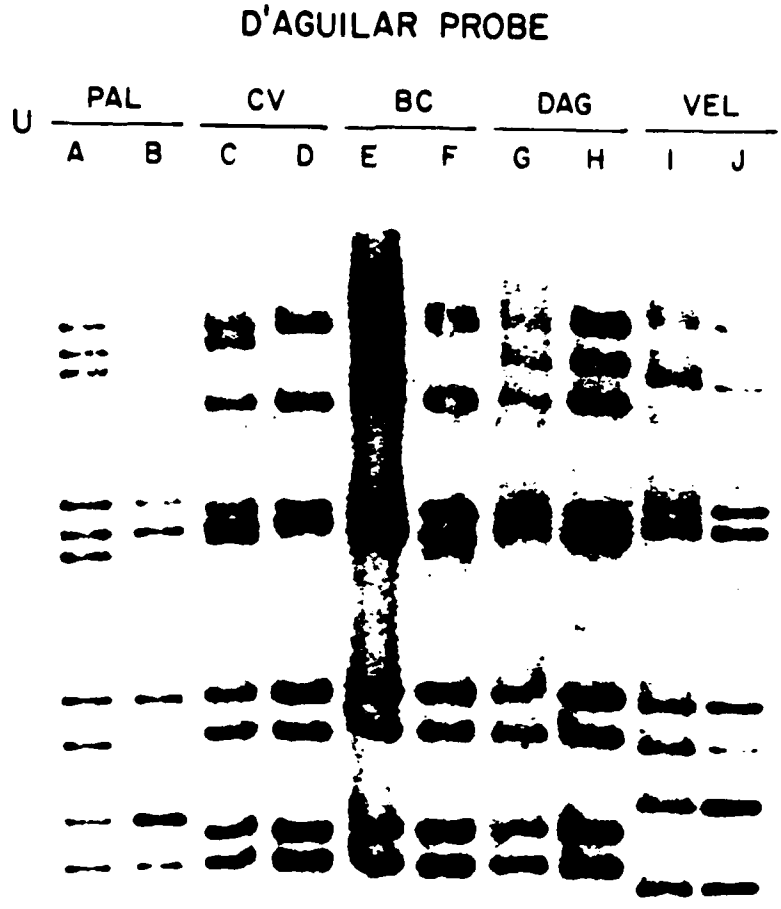


Figure 7. Autoradiogram depicting hybridization of D'Aguilar genomic RNA to the segments of Palyam serogroup viruses. PCp labelled dsRNA from each viruses was electrophoresed in polyacrylamide next to the respective unlabelled dsRNA. Comparison of labelled and unlabelled lanes allows identification of the genes in the unlabelled lanes which hybridized with the probe. In Fig. 7a, lanes are uninfected cellular control (U), labelled (A) and unlabelled (B) Palyam (PAL), labelled (C) and unlabelled (D) CSIRO Village (CV), labelled (E) and unlabelled (F) Bunyip Creek (BC), labelled (G) and unlabelled (H) D'Aguilar (DAG), and labelled (I) and unlabelled (J) Vellore (VEL). In Fig. 7b, lanes are labelled (A) and unlabelled (B) D'Aguilar (DAG), labelled (C) and unlabelled (D) Kasba (KAS), labelled (E) and unlabelled (F) Petevo (PET), labelled (G) and unlabelled (H) Marrakai (MAR), and labelled (I) and unlabelled (J) Abadina (ABA).



D'AGUILAR PROBE

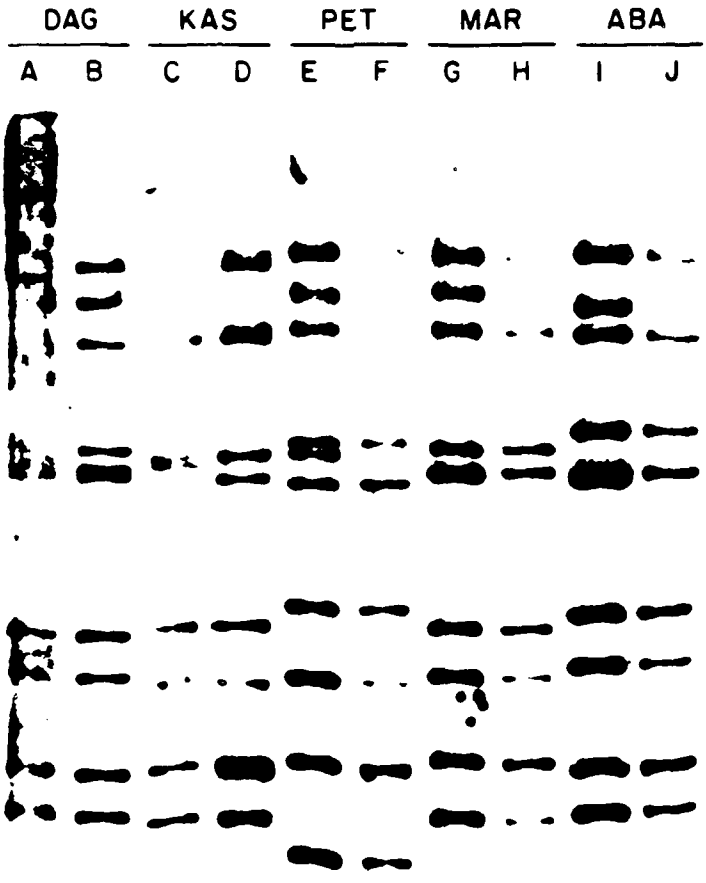
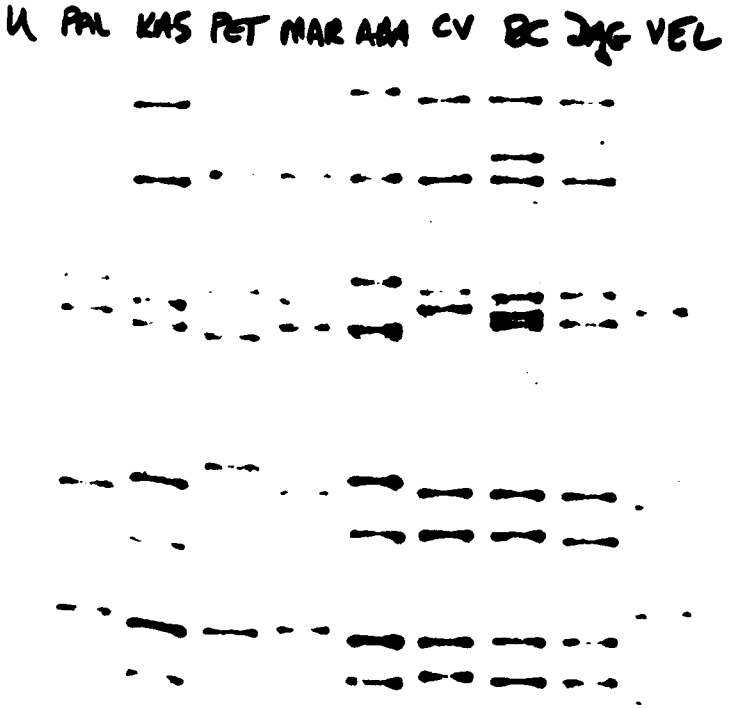
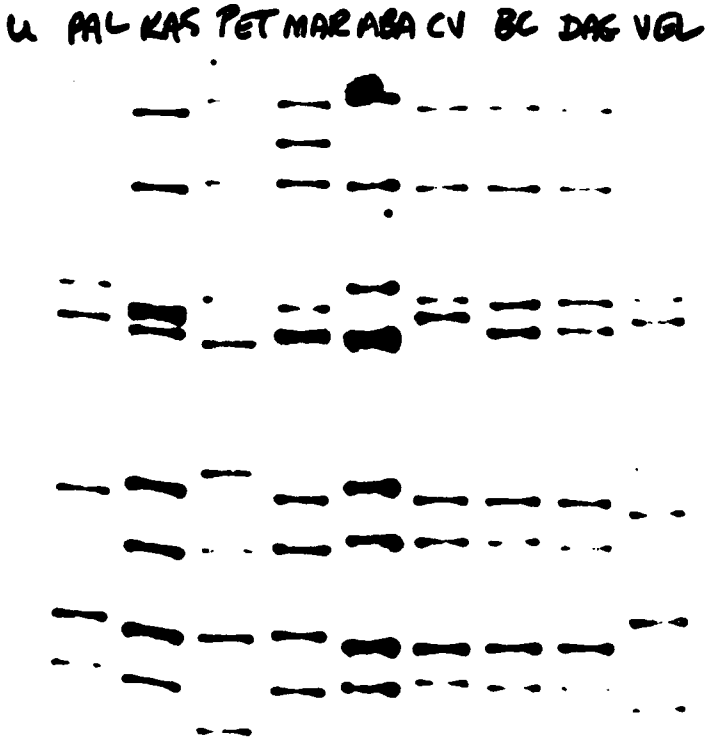


Figure 8. Autoradiograms depicting hybridization of Bunyip Creek (Fig. 8a) and Marrakai (Fig. 8b) genes to their counterparts in the Palyam serogroup viruses. Lanes are designated as described in the legend to Fig. 6. The hybridization in Fig. 8a was performed by removing the Marrakai probe from the membrane (Bodkin and Knudson, 1985b) used in Fig. 8b, and rehybridizing the samples with Bunyip Creek genomic RNA. The position of segment 2 (which does not cross-hybridize in some isolates) may be determined by examining the control labeled lanes for each virus in Figure 7.




U PAL KAS PET MAR ABA CV BC DAG VGL

Genetic Relatedness of Corriparta Serogroup Viruses (H. A. Gonzalez and D. L. Knudson). The genetic relatedness of eight members of the Corriparta serogroup of orbiviruses was determined by reciprocal blot hybridizations of genomic RNA from each virus to the segments of all members of the group. Conserved and variant genes were identified by the degree of cross hybridization between cognate genes of different isolates. The number and identity of genes that were highly related between isolates varied. Three complexes of related viruses were identified within the serogroup based on the number of conserved genes. Four of the viruses, all isolated in Australia, shared six or more of the ten genes and formed one complex of highly related isolates. Another isolate, Acado, was variant in all ten genes and was assigned to a second complex. Eight of the ten genes were conserved between the remaining three isolates; these three viruses formed a third complex. Within each complex, genes 1 and 3 exhibited the greatest number of variants. Genes 6 and 10 also exhibited variants within the complex of Australian isolates. The remaining genes were highly conserved among all members of a complex. No genes appeared to be conserved between viruses in different complexes. Complexes of isolates within a serogroup which are highly related in the majority of the ten genes and much less closely related to serogroup viruses in another complex have not been previously reported in orbiviruses. The identification of complexes within a serogroup demonstrate different levels of relatedness which are not always apparent in taxonomic classifications based on serological data.

The Corriparta isolates (Table 30) exhibit similar but distinguishable polyacryamide gel profiles (Fig. 9); their agarose gel profiles are nearly identical (Fig. 10), confirming the identification of the eight isolates as serogroup members. These results are similar to findings for other orbivirus serogroups (Hrdy et al.,1979; Verwoerd et al., 1979; Knudson et al., 1984; Travassos da Rosa et al., 1984; Bodkin and Knudson, 1985a), and support the use of polyacrylamide rather than agarose gel electrophoresis in preparing the blots since the identity of the different isolates could be confirmed.

The results of the reciprocal blot hybridization experiments were quite different from a similar study of the Palyam serogroup of orbiviruses (Bodkin and Knudson, in press). The majority of Palyam segments are conserved between all isolates in the serogroup. In addition, both variant and unique genes have been identified in the Palyam serogroup. Segment 2, which exhibits unique genes, has been correlated with the gene encoding the major neutralization antigen. Isolates which do not cross-hybridize in gene 2 are distinguishable by neutralization test.

In contrast, results in Figure 11 and Figure 12 indicate that the number of genes that appear to be conserved or variant between isolates depends upon the identity of the probe. For example, most of the genes of the Corriparta prototype appear to be conserved in the three CSIRO isolates (Fig. 11a) but variant between Corriparta and Jacareacanga (Fig. 12b) or Acado (Fig. 12d). Unlike the Palyam serogroup, there are no unique genes in the Corriparta serogroup. Clearly, there are different degrees of relatedness among the Corriparta isolates, reflected in all ten genes, whereas the Palyam viruses are highly related in all but one or two genes. The results of the hybridization experiments are summarized in Table 31.

Corriparta and related viruses. The four Australian isolates--Corriparta, CSIRO 76, CSIRO 109, and CSIRO 134--emerged as a subset of closely related viruses within the Corriparta serogroup. Comparison of the autoradiograms in

Figure 10 shows that when any of the four viruses was used as a probe, RNA from these isolates hybridized more strongly to the probe than did the African or Brazilian isolates, although the number of genes that hybridized strongly and the intensity of the hybridization signal varied.

The CSIRO isolates appear more closely related to each other than to the prototype, and of the three, Corriparta shares more genes with CSIRO 109 than with CSIRO 76 or CSIRO 134. This result is consistent with the isolation data; the CSIRO viruses were isolated in the same area within a two year period, while the prototype was isolated at a different study site over a decade earlier.

Comparison of signal intensities within the lanes indicates that genes 1 and 3 exhibit variants within the complex. For instance, genes 1 and 3 of Corriparta could be distinguished from genes 1 and 3 of each of the CSIRO isolates. However, these genes could not be distinguished in reciprocal blot hybridizations of CSIRO 76 and CSIRO 134.

Genes 6 and 10 also showed variability within the complex, and could be used to distinguish the three CSIRO isolates. Genes 6 of CSIRO 109 could be distinguished from gene 6 of the other two viruses, and gene 10 appeared to be variant in each of the three. The remaining segments, genes 2, 4, 5, 7, 8 and 9, were considered to be conserved within the complex.

Bambari and related viruses. By similar analysis, Bambari, Jacareacanga, and Be Ar 263191 appeared to form a second complex of closely related isolates. The results were harder to interpret than those of the Corriparta complex hybridizations due to variability in the amount of membrane-bound dsRNA and due to differences in the specific activity of the probes. For instance, examination of reciprocal blots of Jacareacanga and Be Ar 263191 (Fig. 12b and c) seems to show that Jacareacanga was not closely related to Be Ar 263191 when Jacareacanga was used as the probe, but Be Ar 263191 was closely related to Jacareacanga when Be Ar 263191 was used as the probe. However, less Be Ar 263191 RNA was available to bind to the probe and the signal was considered to be strong compared to the other lanes such as Acado which contained as much or more material as the probe lane. Again, genes 4, 5, 7 and 8 appeared to be the most highly conserved genes within the complex, and genes 1 and 3 exhibited variants as seen in the Corriparta complex. In contrast with the Corriparta and CSIRO viruses, genes 6 and 10 were conserved within the Bambari complex.

The two Brazilian viruses, isolated within a two year interval, are less related to each other and more related to Bambari, an African isolate, than might be expected. Closely related viruses may occur in distant areas due to the movement of livestock or insects. Conversely, viruses in the same geographical area may be functionally isolated by biological factors such as different host species. The relative importance of geographical origin and time of isolation may be better understood as more members of the Bambari complex are identified.

Acado virus. The remaining isolate, Acado, did not appear to be closely related to any of the other viruses (Fig. 11d). This virus was considered the only known member of a third complex of Corriparta serogroup viruses.

As mentioned earlier, RNA-RNA blot hybridization has been used to determine the genetic relatedness of the Palyam serogroup viruses, with quite different results (Bodkin and Knudson, in press).

Corriparta serogroup viruses also differ from Palyam viruses in host range. As seen in Table 30, most isolates were made from Culex mosquitoes, while Palyam viruses have been isolated from a wide variety of insects and vertebrates. It is likely that the Palyam viruses maintain an arthropod-vertebrate cycle such that the vertebrate immune system exerts selective pressure on viral antigens, producing unique genes. This results in a variety of different serotypes, while the remaining genes are highly conserved. Corriparta viruses do not seem to be exposed to such strong host selection which may be a result of a different life cycle.

The taxonomic division of orbiviruses into serogroups obscures the different levels of relatedness between pairs of isolates within a serogroup. The term complex, as used here, defines subsets of serogroup isolates which are highly related in the majority of the ten genes. The term has been used to categorize the Kemerovo serogroup of orbiviruses based on cross reactivity of the isolates in CF tests; however, the Kemerovo serogroup complexes appear to correspond to complexes identified by blot hybridization experiments (Brown and Knudson, unpublished results).

Not all serogroups may contain more than one complex; the entire Palyam serogroup may be analogous to a single complex within the Corriparta serogroup. Within a complex, as with the entire Palyam serogroup, the majority of genes are conserved. This suggests that unlike the Palyam viruses, the Corriparta isolates may belong to three different species. Gorman (1983) has suggested that the ability to exchange genetic material by reassortment is a better criterion for identifying isolates belonging to the same species than serological cross reactivity. Assessment of the reassortment capability of viruses belonging to the same and different Corriparta serogroup complexes may help to determine whether each complex should be considered a unique species.

These data have been submitted for publication.

Table 30. Corriparta Serogroup Viruses

Virus	Strain	Source	Geographical Origin	Date of Isolation
Corriparta	MRM1	Culex annulirostris	North Queensland Australia	1960
------	CSIRO 76	Culex annulirostris	North Queensland Australia	1974-6
------	CSIRO 109	Culex annulirostris	North Queensland Australia	1974-6
------	CSIRO 134	Culex annulirostris	North Queensland Australia	1974-6
Bambari	Dak Ar B3689	Culex pool	Bambari region Central African Republic	1971
------	Be Ar 263191	Culex declarator	Belem, Para State Brazil	1974
Jacareacanga	Be Ar 295042	Culex (Melanoconion) sp.	Belem, Para State Brazil	1975
Acado	Eth Ar 1846-64	Culex antennatus Culex univittatus neavi	Ilubabor region Ethiopia	1963

Table 31. Genetic Relatedness of Corriparta Serogroup Viruses[a]

Segment	Viruses						
	COR	CSIRO 76	CSIRO 109	CSIRO 134	BAM	Be Ar 263191	JAC	ACA
1	cor[b]	c76	c76	c76	bam	bam	jac	aca
2	cor	cor	cor	cor	bam	bam	bam	aca
3	cor	c76	c109	c76	bam	be ar	jac	aca
4	cor	cor	cor	cor	bam	bam	bam	aca
5	cor	cor	cor	cor	bam	bam	bam	aca
6	cor	c76	cor	c76	bam	bam	bam	aca
7	cor	cor	cor	cor	bam	bam	bam	aca
8	cor	cor	cor	cor	bam	bam	bam	aca
9	cor	cor	cor	cor	bam	bam	bam	aca
10	cor	c76	cor	c134	bam	bam	bam	aca

[a]Membranes containing the genome profiles of each of the isolates were hybridized to 1 ug of [5′-^{32}P] pCp-labeled genomic RNA from each of the other isolates as described in the text.

[b]Variant genes, which differed in degree of cross hybridization to cognates of heterologous isolates, are indicated by a lower case abbreviation of the name of an isolate. In cases where variant genes of two or more viruses cross hybridized strongly, the genes were arbitrarily assigned the designation of one of the viruses involved. No genes were identified as either serogroup genes (genes which cross hybridize strongly in all isolates) or unique genes (those which do not cross hybridize at all to cognate genes of heterologous isolates.

Figure 9. Polyacrylamide gel depicting the resolution of the dsRNA genomes of eight Corriparta serogroup isolates. Genomic RNA was electrophoresed through a Tris-glycine buffered 10% polyacrylamide gel. The gel was stained with ethidium bromide (0.5 ug/ml) for 24 h. Lanes are from left to right uninfected cellular control (U), reovirus type III Dearing strain (R), Corriparta (C), Bambari (B), Jacareacanga (J), Be Ar 263191 (Be), Acado (A), CSIRO 109 (109), CSIRO 134 (134), and CSIRO 76 (76). Segments are numbered 1 to 10 from the top of the gel.

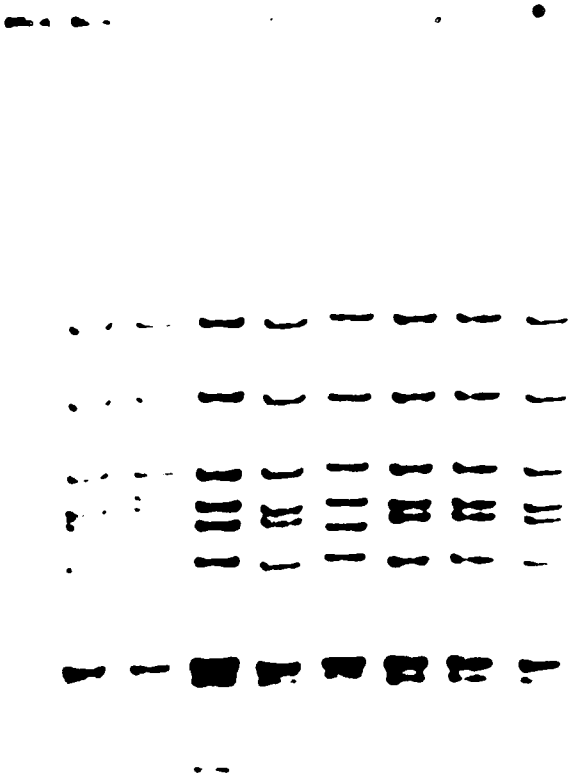


Figure 10. Autoradiogram depicting the resolution of the dsRNA genomes of the Corriparta isolates by electrophoresis through a 1% agarose gel. Lanes are from left to right Corriparta (C), Bambari (B), Jacareacanga (J), Be Ar 263191 (Be), Acado (A), CSIRO 109 (109), CSIRO 134 (134), and CSIRO 76 (76).

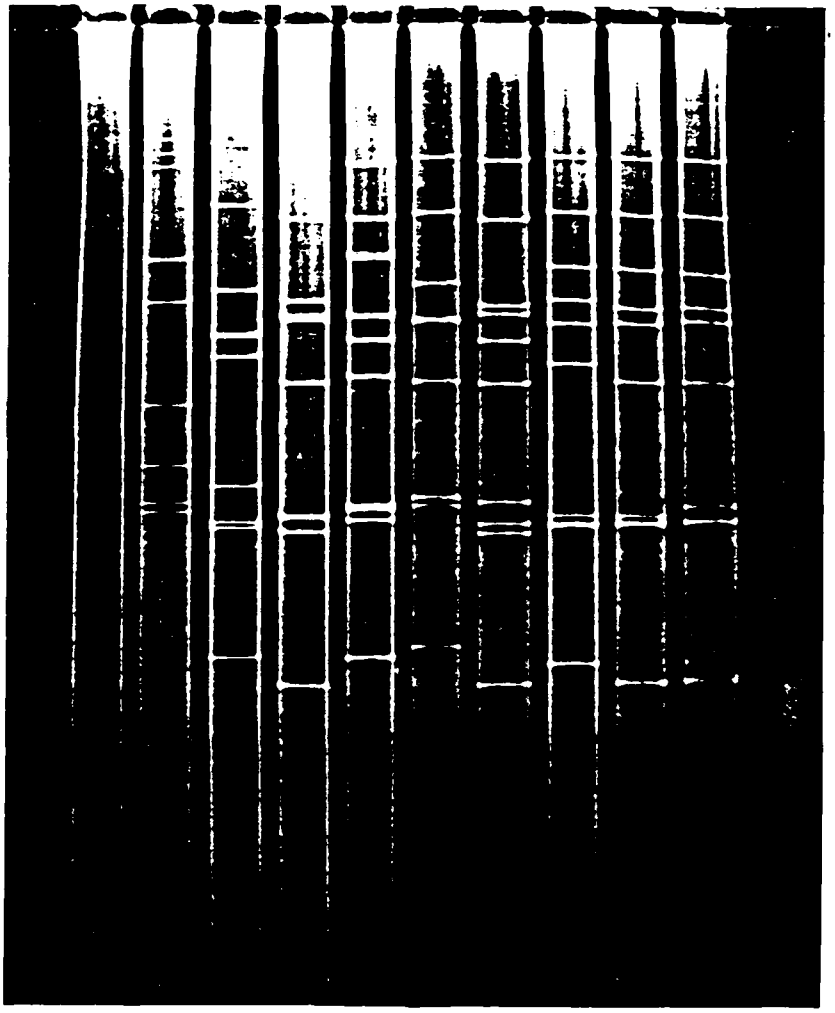
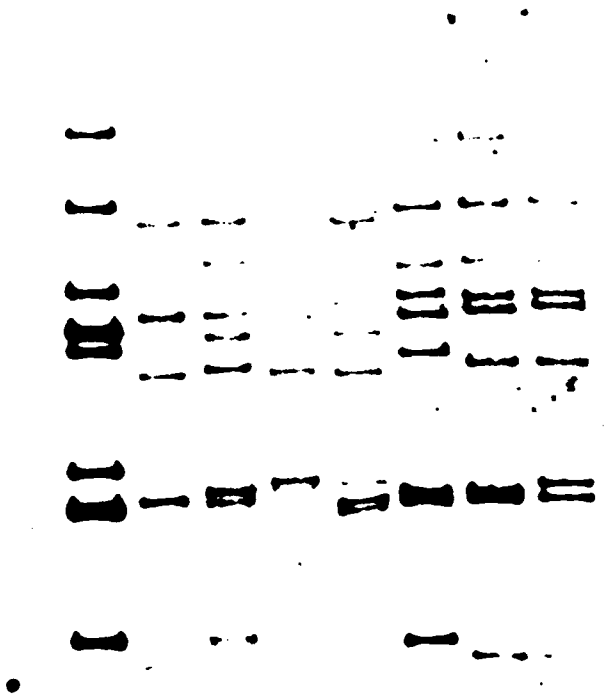
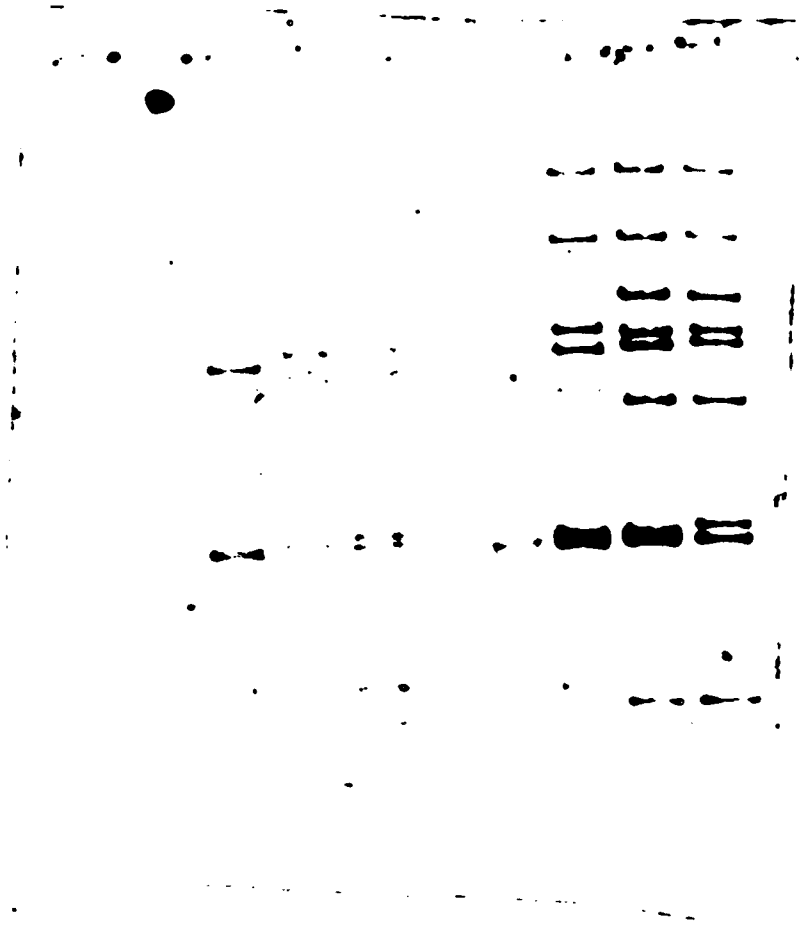
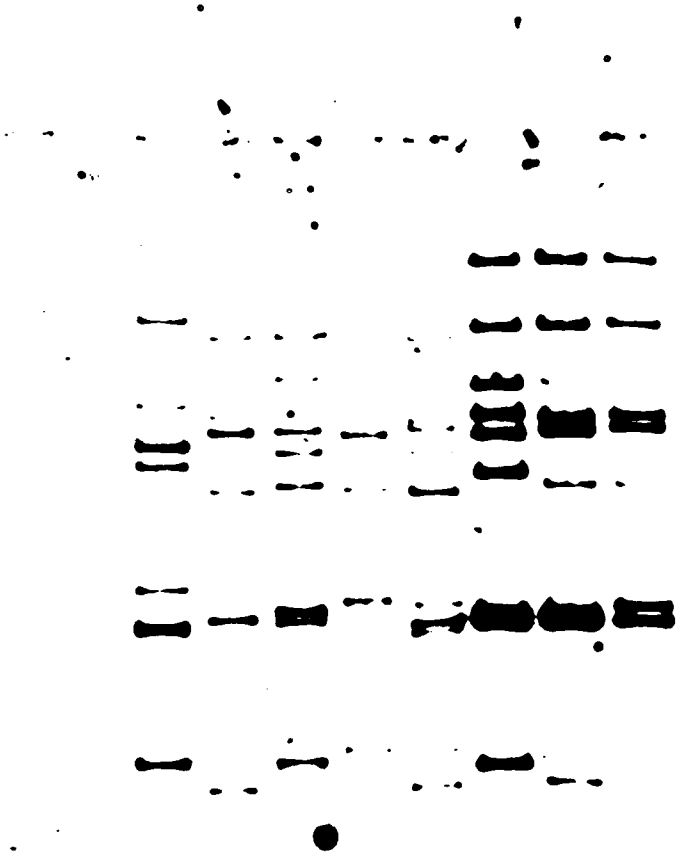


Figure 11. Autoradiogram depicting hybridization of probe genes to counterparts in the Corriparta serogroup viruses. Total genomic dsRNA was end labeled with [5'-^{32}P]pCp and hybridized to the genomic profiles of the serogroup members which were transferred from polyacrylamide gels to a Zeta-Probe membrane. Lanes are as in Figure 9. The membrane was hybridized with Corriparta (a) CSIRO 76 (b) CSIRO 109 (c) and CSIRO 134 (d).







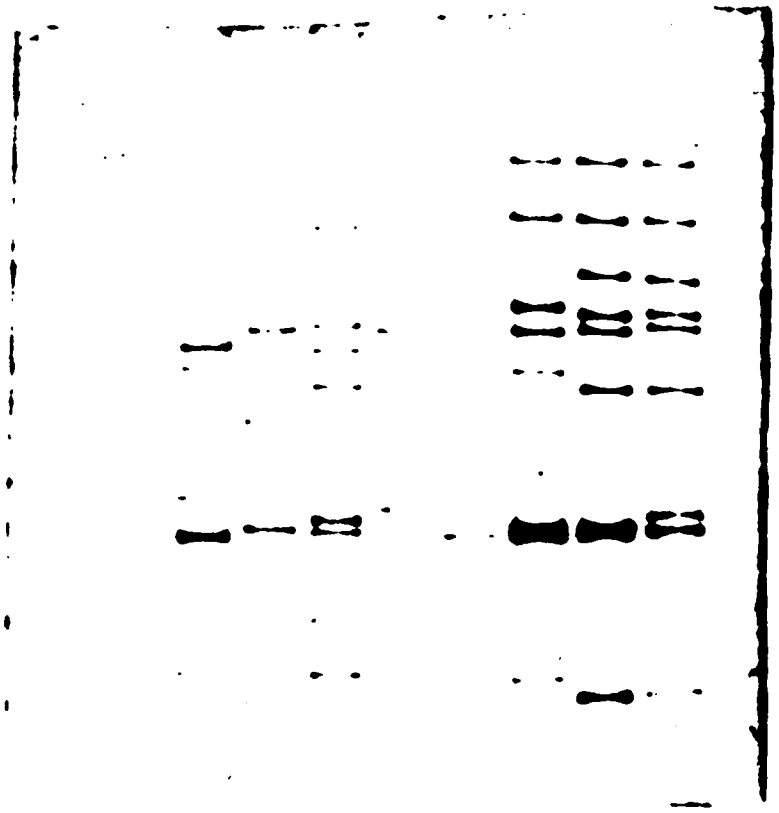
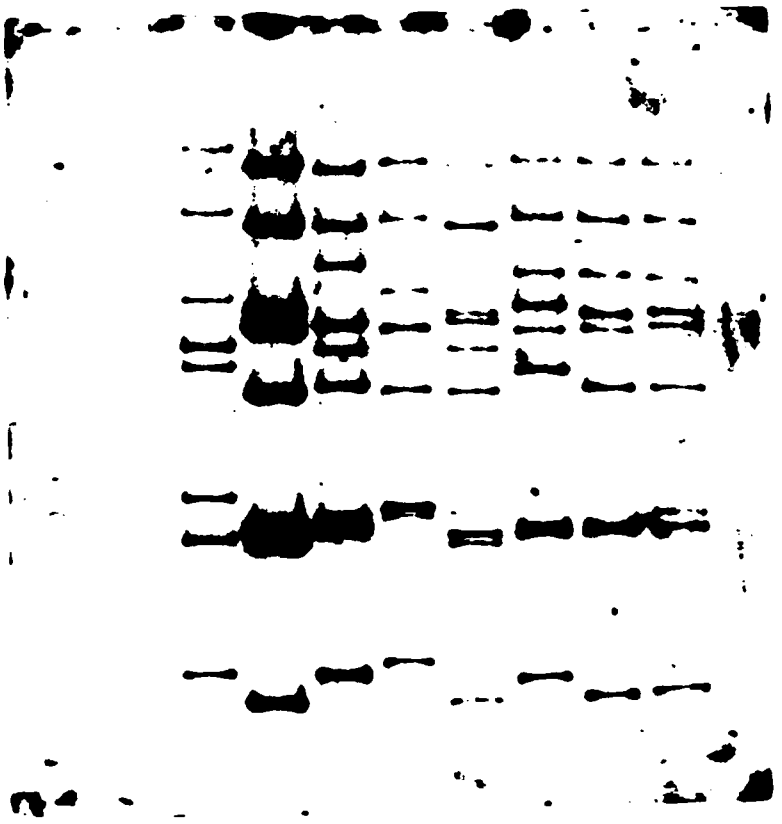
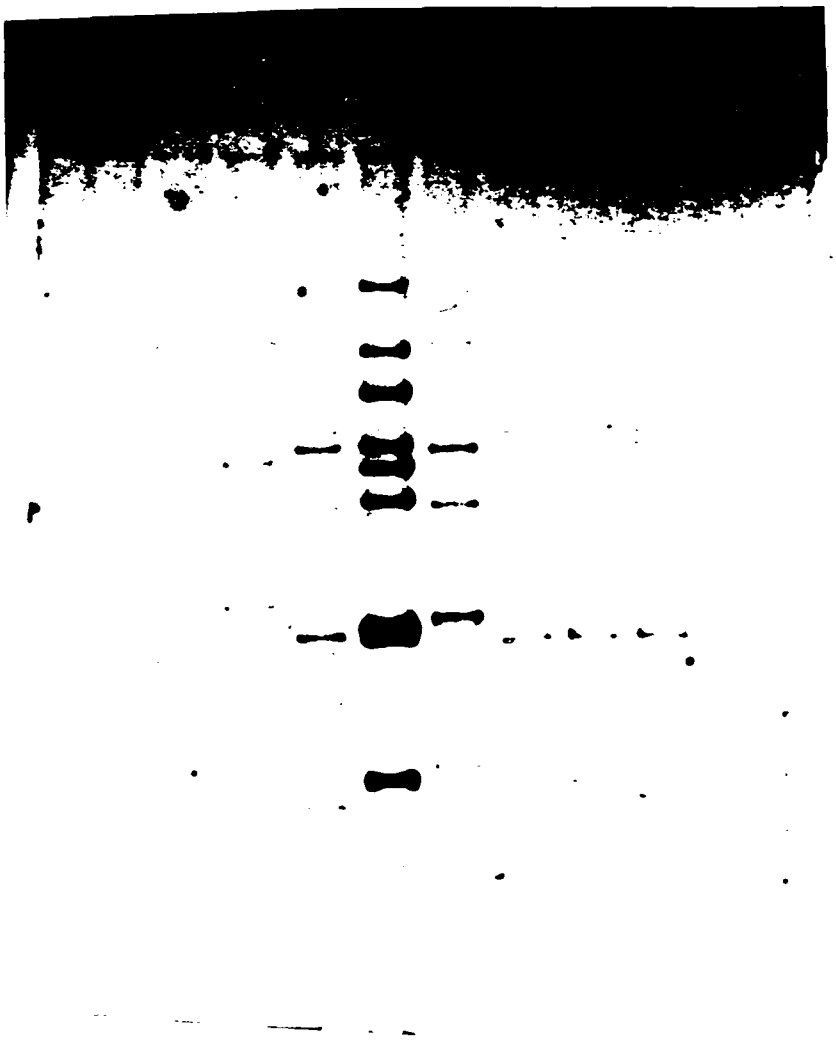
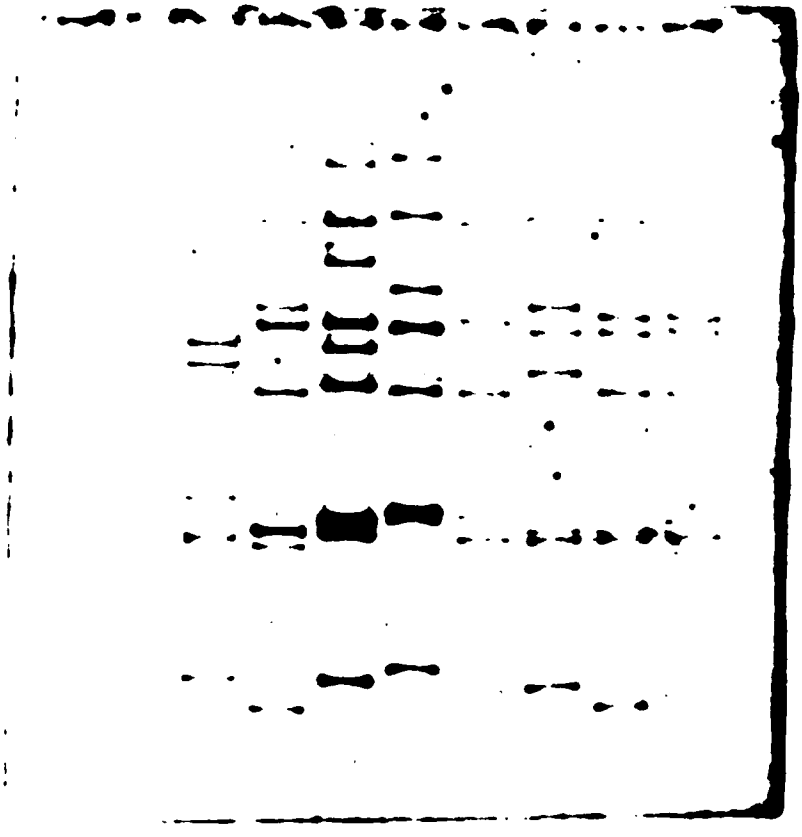
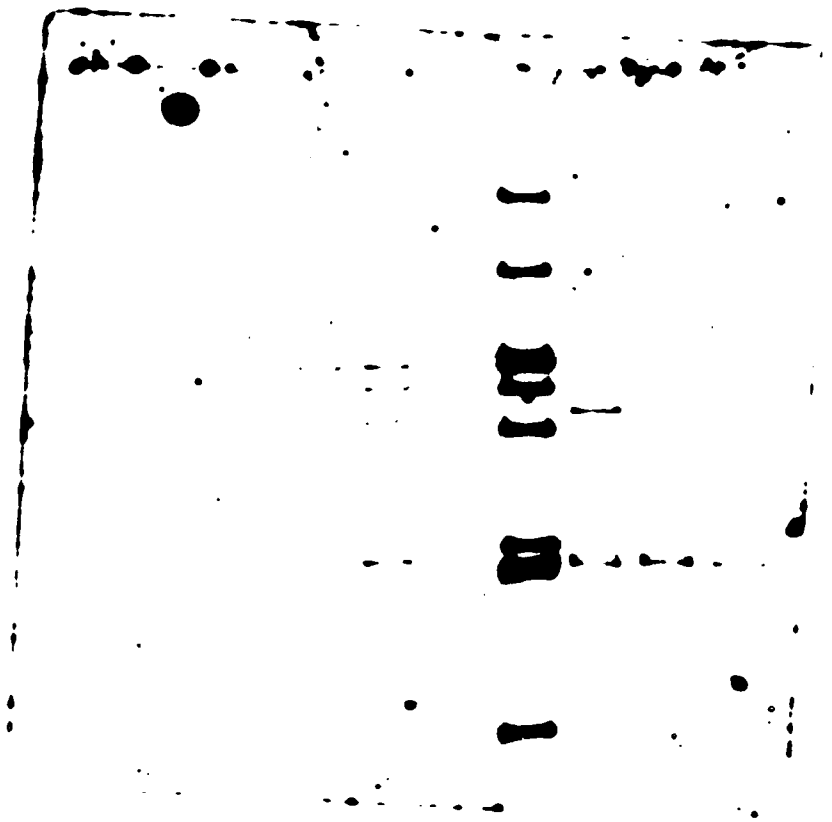


Figure 12. Autoradiogram depicting hybridization of probe genes of counterparts in the Corriparta serogroup viruses. Lanes are as in Figure 9. The membrane was hybridized with Bambari (a) Jacareacanga (b) Be Ar 263191 (c) and Acado (d).









Genetic Relatedness of Colorado Tick Fever Virus Serogroup Isolates by RNA-RNA Blot Hybridization (D.K. Bodkin and D.L. Knudson). The genetic relatedness of members of the Colorado tick fever (CTF) serogroup viruses was examined by RNA-RNA blot hybridization. Stringent conditions for hybridization (52°C, 50% formamide) were chosen in order to maximize the differences between the isolates examined. All 12 genes of 11 isolates examined exhibited nucleic acid sequence homology with their corresponding (cognate) gene when either CTF-FMA or CTF-18 was used as probe. Genes 4 and/or 6 of certain isolates showed less relatedness to their cognates in CTF-FMA than did other genes. For example, the level of cross-hybridization between genes 4 of CFT-FMA and CTF-18 was lower than the level of cross-hybridzation seen in the other genes of these two isolates (Table 32).

CTF-FMA was isolated in 1943, while the other strains were isolated between 1964 and 1977. Yet, the degree of sequence divergence between cognate genes is minor when compared with the data for other Orbivirus serogroups. In general, the CTF serogroup viruses represent a genetically homogeneous group of viruses. The significance of these findings will be discussed in light of the biologic data that has accumulated on these agents.

Table 32. Summary of CTF Gene Relatedness

	CTF Isolates									
Segment	Florio (FMA)	64V1078	76167	76181	R6205	76170	71V1	76193	SS-18
1	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
2	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
3	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
4	fma	ss	ss	ss	fma	fma	ss	ss	fma	ss
5	ctf	fma	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
6	fma	?	?	?	?	?	?	?	?	ss
7	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
8	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
9	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
10	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
11	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf
12	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf	ctf

ctf = common genes
fma = CTF Florio FMA
ss = CTF SS-18
? = Not CTF Florio FMA or CTF SS-18

These data have been submitted for publication.

SDS treatment of antigen for use in ELISA (J. Oprandy, T. Schwan, and A.J. Main). We tested the use of sodium dodecyl sulfate (SDS) to inactivate virus-infected BHK-21 cells used as antigen with an increase in sensitivity in a direct ELISA. SDS has long been used to dissociate (therefore inactivate) viruses and large proteins into their individual polypeptide chains. These polypeptides retain their antigenicity and can be employed in immunologic assays. SDS is a low cost, non-toxic chemical which has no safety requirements regulating its use. Sensitivity of ELISA detection of viral antigens is increased by a reduction in background and the release of more soluble antigen from infected cellular material. There is increased binding of proteins of polystrene ELISA plates. Most importantly, there will be increased safety in handling biohazardous material.

There are advantages to this simple and rapid technique. High background in assays may be reduced, lower yields of virus might also be detected. Biopsy material may be quickly dissociated and assayed. Desorbing activity of stool samples by proteolytic enzymes may be inhibited. Finally, use of potentially hazardous, logistically difficult or expensive reagents or protocols for the treatment of microbial antigens may be facilitated.

Stocks of Great Island (CanAr 41) and Cape Wrath (ScotAr 20), were grown in monolayer cultures of BHK-21 cells. Tissue culture harvest was adjusted to 0.1%, 0.5% or 1.0% SDS concentration by addition of 20% SDS in PBS and then incubated at 37C for 2h or 100C for 5 minutes. Two-fold serial dilutions from 1:25 to 1:1600 were made in PBS and used to coat plates in the direct ELISA. Each assay was performed for both viruses in duplicate plates on two separate occasions. Ascitic fluid dilutions wre determined by block titrations. Controls were normal mouse ascitic fluid in lieu of hyperimmune mouse ascitic fluid, and uninfected BHK-21 TC in lieu of virus infected BHK-21 TC.

It was found that a concentration of 1.0% SDS in the coating mixture interfered with the antigen detection ELISA. For this reason, material was treated with SDS and then diluted in PBS. Titres of virus-infected TC material were 4.07 and 4.20 log PFU/ml for GI and CW viruses respectively. ELISA results for both viruses were equivalent.

Results shown in figures 13 and 14 are expressed in positive/negative control (P/N) absorbance values. Negative controls were identically treated uninfected TC material. Estimation of background was determined by mean +3 SD of negative controls. P/N values increased with SDS treatment (Fig. 13). Optimal treatment was 1.0% SDS at 37°C for 2 h. Treatment at 1.0% SDS was significantly different from untreated ($P < 0.001$) and 0.1% SDS treated material ($P < 0.01$). Treatment at a concentration of 0.5% SDS was not significantly different from 1.0% SDS treated TC ($P > 0.1$). Boiling SDS treated material, while not optimal, did significantly ($P < 0.001$) improve ELISA sensitivity. 0.1% SDS treatment was not significantly different from untreated material ($P > 0.05$).

A comparison of untreated versus 0.5% and 1.0% SDS-treated TC antigen in the direct ELISA (Fig. 14) showed that the majority of data points were positive for treated and negative for untreated material. Specifically, untreated TC material titered to 1:100 when probed with a constant dilution of ascitic fluid while 0.5% and 1.0% SDS-treated TC-material titred to 1:800, a difference of 8-fold greater. This indicates an increased sensitivity with SDS treatment. OD

values for negative antigen controls (Fig.15) averaged 3 times higher in the untreated material than in treated (1.0% SDS) material. This reduction in background contributed to the increased P/N ratio of the treated samples. While OD values of the untreated negative antigen controls decreased slightly with dilution, OD values for SDS-treated negative antigen increased slightly with dilution.

Virus inactivation with SDS treatment was assayed by attempting to infect BHK-21 cells with 0.01% SDS treated virus infected TC. This material was first diluted 1:10 with sterile culture medium. CPE was observed in untreated virus infected cells within 24 h post-infection and total cell destruction in 48 h. SDS treated virus infected cells showed no CPE.

The treatment of tissue culture harvest with 1.0% SDS at 37°C for 2 h before use as antigen in ELISA resulted in a decrease of background. Binding of antigens from virus-infected cell culture material was at least as efficient in SDS treated samples. The high background observed in untreated TC antigen was greatly reduced by SDS treatment.

Treatment of TC material at 0.5% SDS or 1.0% SDS at 37°C was found to be optimal for antigen detection ELISA. A concentration of 0.1% SDS had little or no effect. Results for SDS treated antigen boiled for 5 min were not optimal. While a 0.5% SDS concentration for antigen treatment was not statistically different, P/N ratio's for 1.0% SDS treated material were slightly higher. This may be relevant for optimizing final SDS concentration in coating material and material used in other ELISA test systems. Orbiviruses are double-shelled non-enveloped RNA viruses. Optimal SDS treatment for enveloped viruses may be milder.

A high residual concentration of SDS did interfere in subsequent ELISA steps; for this reason, TC material should be treated and then diluted in buffer (at room temperature). It was observed that coating overnight could be done at room temperature or at 4C; in addition, significantly shorter coating times were achieved with antigen bound with SDS. SDS, being a negatively charged molecule, has an affinity for polystyrene ELISA plates. Any residual SDS in diluted coating antigen, after washing 3 times, did not interfere with binding specificities or enzymatic reactions in later steps in the test. The effect of prolonged storage of treated antigen or coated plates is undetermined.

The direct ELISA test for Kemerovo serogroup orbiviruses is complex specific. SDS treatment did not alter this specificity. The level of sensitivity, however, was increased 8-fold. This was due, in part, to a reduction of OD value for negative control antigen which increased P/N ratios. OD values for specific antigen were similar for treated and untreated material though poor antigen binding to plates in some untreated TC preparations was observed.

Difficulties in antigen detection ELISA are currently addressed using a number of protocols. High background in uninfected cell controls has been limited by treating samples with 10% formalin. Infectious Lassa fever virus, used as antigen, is inactivated with B-propiolactone or gamma-irradiation. Advantages of SDS treatment of antigen for ELISA are many. It is fast, easily performed and uses a widely distributed chemical which in itself presents no

health risk. This is in contrast with other agents, formalin, gamma-irradiation and beta-propiolactone. SDS treatment increased the level of sensitivity and binding efficiency in the ELISA. SDS treatment should readily inactivate viruses and other microorganisms thus rendering them safer to handle and more easily employed as reagents in ELISA.

SDS was applied to suspensions of triturated Argas sp.n. and Ixodes uriae pools to try to increase sensitivity for viral antigen detection when tested by capture and direct ELISA. Tick pools had been tested previously for virus by inoculation into Vero and/or BHK cells. Suspensions of 10 Mono Lake positive Argas pools and 17 negative pools were aliquoted into two samples, 1 of which was treated with 1.0% SDS (2 hrs at 37C, then held overnight at room temperature). Three replicates of each treated and untreated pool was tested for Mono Lake virus antigen using capture ELISA. The ELISA protocol included coating the 96 well Immulon plate with mouse anti-Mono Lake, blocking, SDS treated and untreated tick suspension, rat anti-Mono Lake, mouse anti-rat horseraddish peroxidase conjugate, and hydrogen peroxide - ABTS substrate.

For the 10 tick suspensions positive by tissue culture, only one SDS-treated and two untreated suspensions were positive using capture ELISA (absorbance greater than the mean absorbance plus 3 SD of negative pools). Also, the mean absorbance for SDS treated pools that were negative tissue culture was twice the mean absorbance for untreated pools (0.55 and 0.245, respectively). Therefore, SDS treatment did not increase sensitivity for antigen detection and it also increased the background absorbance.

Another attempt to increase sensitivity was done by decreasing the concentration of SDS to 0.5% and then serially diluting the suspensions with PBS to a final dilution of 1:1600. Again, there was no increase in sensitivity or decrease in background. With these two methods, SDS treatment of tick suspensions did not increase the sensitivity of ELISA for antigen detection.

Cross reactions of Chenuda complex antigens using capture ELISA (T.Schwan). A capture ELISA to detect Mono Lake virus antigens was used to examine its cross reactivity with other viral antigens belonging to the same antigenic complex (Chenuda) of the Kemerovo serogroup. The sequential protocol for the capture ELISA included coating 96 well Immulon plates with mouse anti-Mono Lake, blocking, antigen, rat anti-Mono Lake, mouse anti-rat horseradish peroxidase conjugate, and hydrogen peroxide - ABTS substrate. Chenuda complex sucrose-acetone extracted suckling mouse brain antigens used were Mono Lake, Huacho, Sixgun City, and Chenuda. Antigens of Great Island, a member of a different antigenic complex, and normal mouse brain were also used to examine complex specificity and as negative controls, respectively. All antigens were treated in serial two-fold dilutions from 1:10 to 1:320. Absorbances greater than the mean absorbance plus 3 SD for normal mouse brain replicates at each dilution were considered positive.

Antigens of all Chenuda complex viruses tested were positive at 1:10 (Fig. 16), while antigens of Great Island virus were negative. Therefore, there is cross reaction with antigens of members of the Chenuda complex when using the capture ELISA to detect Mono Lake virus antigen. Antigen of Great Island virus was negative, indicating that the assay is specific for the Chenuda antigenic complex. Results indicate also that Huacho antigen is most cross reactive in the Mono Lake assay, being positive at all dilutions tested. This agrees with

complement-fixation and neutralization data which indicate that Huacho virus is closely related to Mono Lake virus.

Comparison of virus/antigen detection assays for Mono Lake virus in Argas ticks (T.Schwan). Three assays were used to compare their effectiveness at isolating or detecting Mono Lake virus in Argas sp.n. ticks collected from islands in Mono Lake, California. A total of 200 female Argas ticks, 50 from each of four islands, were triturated individually, aliquoted into three samples, frozen (-70C), and later tested by three assays: Vero cells, BHK cells, and capture ELISA for antigen detection. Monolayers of Vero cells and BHK cells were grown in 24 well tissue culture plates and each pool was inoculated into two wells. Cells with cytopathic effect were passaged and then tested by ELISA for Mono Lake antigen. To test the original suspension by capture ELISA, the sequential protocol included coating the 96 well Immulon plate with mouse anti-Mono Lake (overnight at 4C), blocking, tick suspension (1 hr at 37C, then overnight at 4C), rat anti-Mono Lake, mouse anti-rat horseradish peroxidase conjugate, hydrogen peroxide - ABTS substrate, and spectrophotometric absorbance at 414 nm. The same protocol was used to test tissue culture fluids for Mono Lake virus except that the incubtion of antigen was for only 1 hr at 37C. All other incubations after coating were also done for 1 hr at 37C.

From 200 ticks, 5 strains of Mono Lake virus were isolated in BHK cells, 4 of these 5 were also isolated in Vero cells, while only 2 were positive by capture ELISA (Table 33). There were no pools positive by ELISA but negative in tissue culture. These results indicate that tissue culture is a more sensitive assay for Mono Lake virus then is capture ELISA and that the true infection rate (2.5%) in female ticks is low.

Sensitivity of capture ELISA for antigen detection (T. Schwan). Ten isolates of Mono Lake virus were passaged in Vero cells, aliquoted, and tested by capture ELISA and plaque assay to determine virus titer necessary for antigen detection. Tissue culture fluids of 10 isolates were serially diluted two-fold from 1:10 to 1:5120 and tested for antigen (2 replicates each) using the capture ELISA described above. The same isolates were also tested in serial ten-fold dilution (two replicates) for plaque formation in Vero cells using tragacanth and agar-neutral red overlays in 24 well plates.

Using capture ELISA, endpoints for the 10 isolates ranged from 1:20 to 1:320; however, plaque-reduction titrations showed these same isolates ranging from 3.48 to 4.95 dex plaque-forming units per 0.1 ml (the same volume used in the capture ELISA) (Table 34). Therefore, approximately three logs of Mono Lake virus are needed for antigen to be detected by capture ELISA.

Table 33. Isolation of Mono Lake virus from 200 female Argas ticks.

Island	no. tested	number of female ticks positive: Vero cells	BHK cells	ELISA	Total
Little Tahiti	50	1 (113)*	1 (113)	1 (113)	1
MacPherson D	50	1 (164)	1 (164)	0	1
Little Norway	50	1 (246)	2 (245,246)	1 (245)	2
Spot	50	1 (286)	1 (286)	0	1
Total	200	4	5	2	5
Infection Rate		2.0	2.5	1.0	2.5

* number positive (lot number)

Table 34. End-points for 10 isolates of Mono Lake virus titrated by plaque assay and by capture ELISA.

ISOLATE	PLAQUE-FORMING UNITS/0.1ml (dex)	ELISA/0.1ML
CalAr 861	3.48	1:20
CalAr 1416	4.0	1:160
CalAr T-2	4.95	1:320
CalAr T-7	3.93	1:20
CalAr S-11	4.60	1:320
CalAr S-18	4.0	1:80
CalAr N-23	4.0	1:80
CalAr N-26	4.38	1:80
CalAr M-32	4.60	1:80
CalAr M-34	3.95	1:80

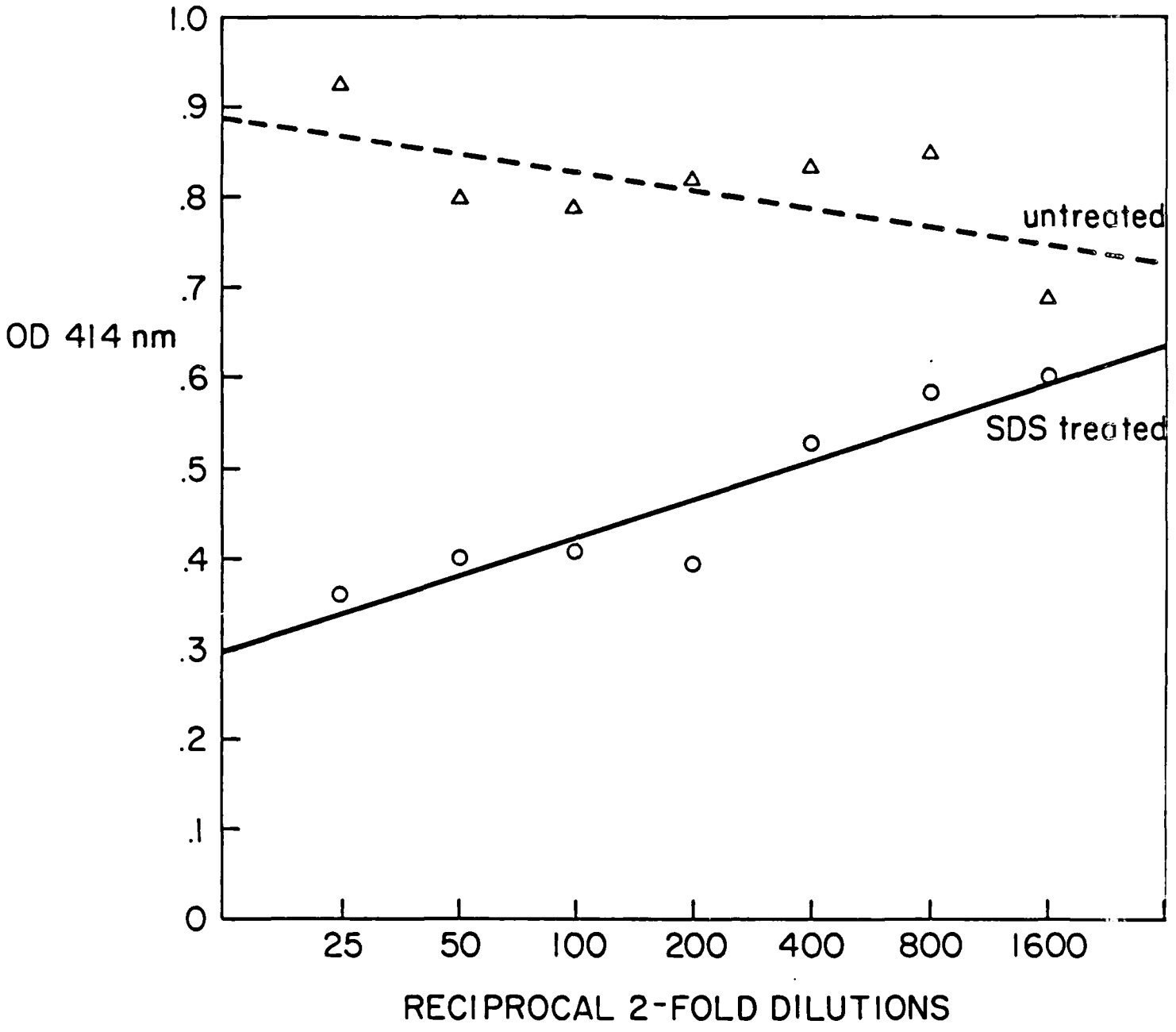


Figure 13 Relationship of OD values for untreated and 1.0% SDS treated negative tissue culture material. Each data point represents a mean of 4 samples.

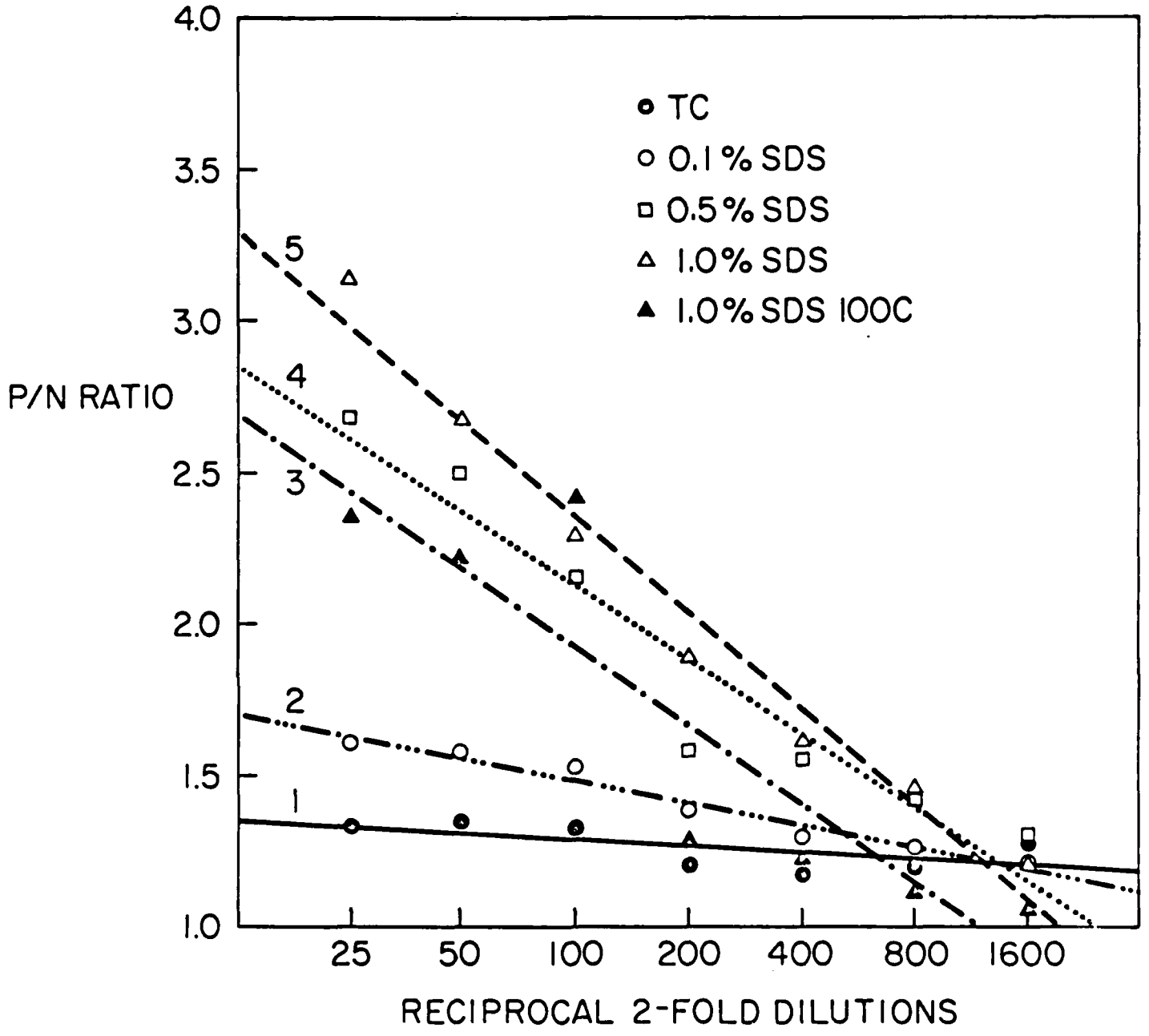


Figure 14. Relationship between ELISA P/N ratio and dilution of tissue culture material. Regression lines are: 1) untreated tissue culture fluid, 2) tissue culture treated at 0.1% SDS, 3) tissue culture treated at 1.0% SDS, 100°C, 4) tissue culture treated at 0.5% SDS, and 5) tissue culture treated at 1.0% at 37°C. Sample size for each regression is 28.

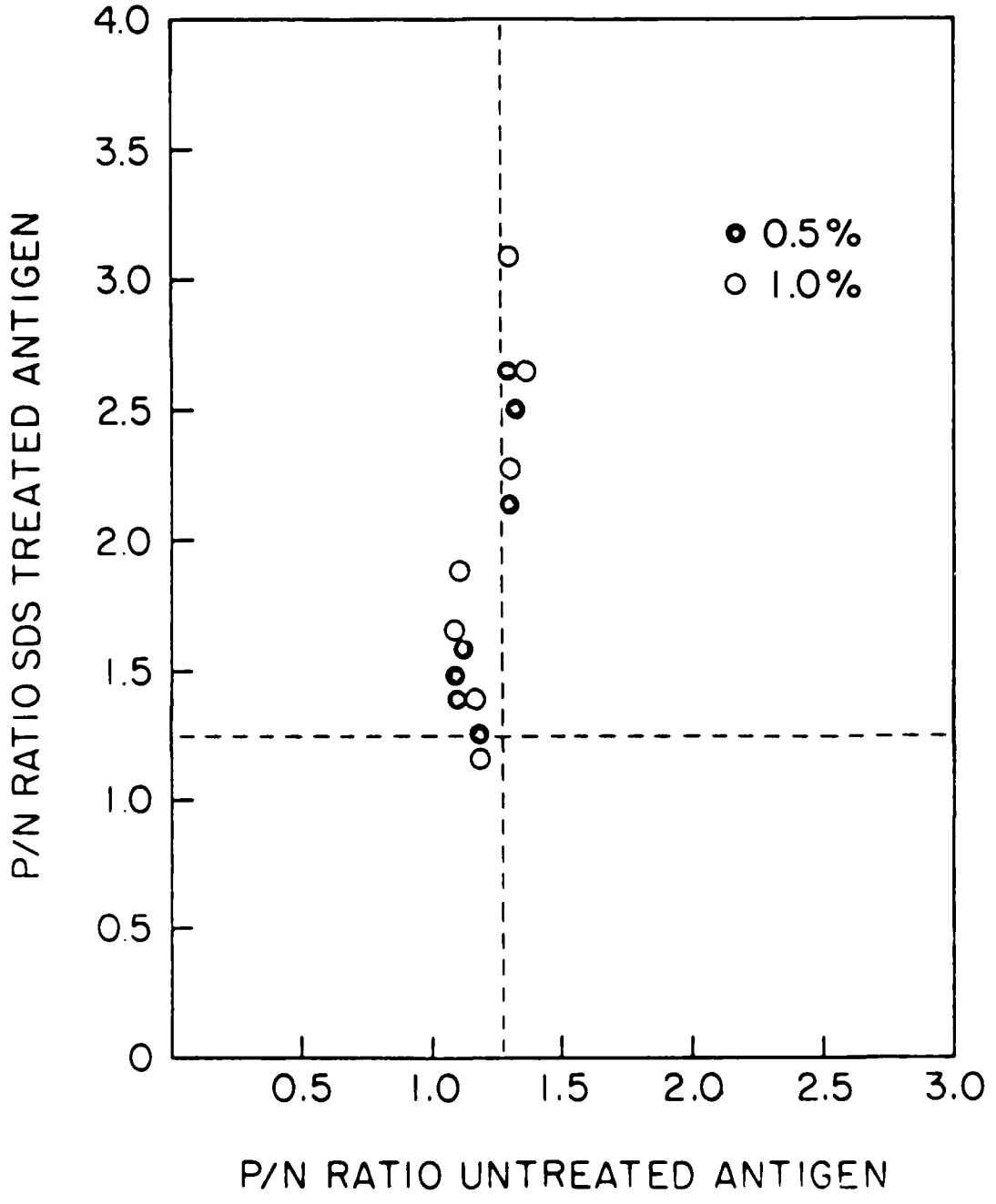


Figure 15. Comparison of P/N values for 0.5% and 1.0% SDS treated versus untreated tissue culture material. Dashed lines represent calculated level of background. Data points represent a mean of 4 samples.

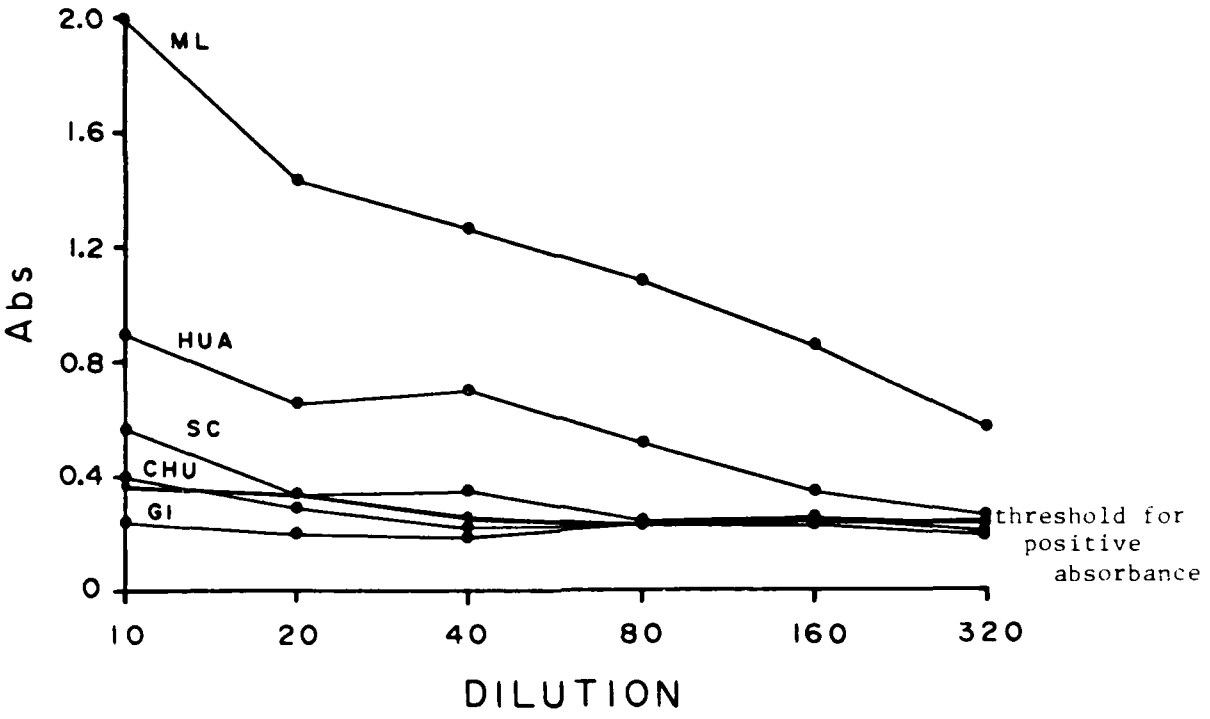


Figure 16 Cross reactions of Chenuda complex viral antigens in capture ELISA for Mono Lake virus.

Development of the ELISA for Crimean-Congo hemorrhagic fever (R.E. Shope, J. Meegan, Rob Scott, and Tom Ksiasek). The ELISA for CCHF was developed previously using rabbit serum to coat the solid phase and mouse brain sucrose-acetone extracted antigen. The test was taken to NAMRU-III, Cairo where it was applied to sera of sheep from Sudan and Somalia. In an attempt to determine the correlation with the HI test, some positive and some negative sheep sera were coded and retested blindly by HI using sera extracted with acetone and with the reference acetone-ether technique. There were 2 inconsistencies between the HI techniques. Somalia 1393 and Sudan 7429 were HI positive with the acetone extraction technique and HI negative with the acetone-ether technique. In addition, Somalia 1388 inhibited antigen in both HI techniques, but not completely, and therefore was read as negative. The results comparing the acetone-ether HI and the ELISA are shown in Table 35. There is correlation, but not complete. The non-correlation involves primarily the 3 sera which were in question in the HI test. The indication from the present experiment is that the ELISA with sheep sera may be more reliable than the HI test, especially the HI using acetone-ether extraction of sera.

Table 35

Correlation of CCHF HI and ELISA using sera of sheep from Somalia and Sudan

Serum	HI titer	ELISA OD
Somalia 1386	80+	.28
Somalia 1387	0	.03
Somalia 1388	0	.40
Somalia 1390	0	.00
Somalia 1391	0	.00
Somalia 1392	40	.99
Somalia 1393	0	.68
Somalia 1394	20	.51
Somalia 1402	0	.06
Sudan 2751	80+	.31
Sudan 7415	40	.61
Sudan 7416	80+	.67
Sudan 7418	20	.28
Sudan 7426	10	.37
Sudan 7427	0	-.01
Sudan 7428	10	.07
Sudan 7429	0	.19
Sudan 7430	40	.72
Sudan 7431	20	.10
Sudan 7432	40	1.09

Development of broad flavivirus monoclonal antibodies (R.E. Shope). Dr. Udy Olshevsky of the Israel Institute for Biological Research referred 12 West Nile monoclonal antibodies for determination of the HI reactivity with a battery of flavivirus antigens. Table 36 shows the reactions with a wide range of flaviviruses of the YARU collection. Numbers 4, 6, 9, 10, 11, and 12 were broadly cross-reacting and relatively high titered. Monoclonal antibody number 10 reacted with all 26 antigens tested, the titers ranging from 1:10,000 to 1:160,000.

Table 36

HI test results of West Nile monoclonal antibodies with flaviviruses

	Antibodies											
	1	2	3	4	5	6	7	8	9	10	11	12
Sepik	0	0	0	4,000	0	8,000	0	0	16,000	40,000	16,000	20,000
Zika	0	0	0	8,000	0	16,000	0	0	32,000	160,000	16,000	40,000
Apoi	0	0	0	2,000	0	4,000	0	0	16,000	40,000	8,000	10,000
Dakar bat	0	0	0	2,000	0	4,000	0	0	16,000	20,000	8,000	10,000
Dengue-1	0	0	0	4,000	0	4,000	0	0	16,000	40,000	16,000	10,000
Ilheus	0	0	0	4,000	0	8,000	0	0	32,000	80,000	16,000	20,000
Japanese E.	0	0	0	1,000	0	8,000	0	0	32,000	80,000	16,000	5,000
Israel TME	0	0	0	1,000	0	8,000	0	0	16,000	20,000	8,000	20,000
Wesselsbron	0	0	0	4,000	0	16,000	0	0	64,000	40,000	8,000	20,000
Edge Hill	0	0	0	500	0	2,000	0	0	4,000	10,000	1,000	10,000
Kunjin	>40	0	>40	2,000	>40	8,000	>40	>40	32,000	40,000	8,000	20,000
Saumarez R.	0	0	0	4,000	0	16,000	0	0	32,000	80,000	16,000	40,000
Spondweni	0	0	0	4,000	0	8,000	0	0	16,000	40,000	8,000	20,000
Bussuquara	0	0	0	2,000	0	16,000	0	0	16,000	10,000	16,000	10,000
Murray Val.	0	0	>40	2,000	0	4,000	0	0	1,000	40,000	16,000	40,000
Dengue-3	0	0	0	16,000	0	16,000	0	0	32,000	160,000	16,000	10,000
Rio Bravo	0	0	0	8,000	0	32,000	0	0	32,000	80,000	8,000	40,000
Kadam				1,000		4,000			8,000	40,000	2,000	10,000
Jutiapa				1,000		4,000			4,000	20,000	2,000	20,000
Banzi				2,000		8,000			32,000	40,000	8,000	20,000
St. Louis				4,000		8,000			32,000	80,000	8,000	40,000
Powassan				1,000		1,000			4,000	10,000	2,000	10,000
Kokobera				4,000		8,000			16,000	80,000	16,000	40,000
Saboya				250		2,000			8,000	20,000	4,000	5,000
West Nile*	40,000	80	40,000	5,000	40,000	40,000	640	40,000	40,000	40,000	20,000	40,000
y.f.	80	0	320	10,000	80	20,000	-	40	20,000	20,000	20,000	20,000

*Studies done in Israel.

Anti-idiotypic Antibody in the Diagnosis of Dengue 2 Virus Infections
(G. Tignor, R. Cedeno, T. Burrage, L. T. Figueiredo, R. Shope)

INTRODUCTION.

After immunization across xenogenous, allogenic, or syngeneic barriers, anti-idiotypic antibodies are obtained which are heterogeneous in regard to their functions and specificity. The network theory of Jerne is based on the dual character of Ig molecules which consist of two distinct entities: combining site, or paratope, which interacts with antigens (epitopes), and idiotopes, which can be recognized by gene products of other B cell clones. Anti-idiotypic antibodies can be classified into four major categories.

One class of anti-Id antibodies represents a category of anti-Id antibodies which carries the internal image of the antigen, i.e., their idiotypes cross-react with the foreign epitopes. These anti-Id antibodies mimic the antigen binding. Because the chemical structure of the paratope and the idiotope is generally very different from the structure of the antigens, the similar three-dimensional structure of this anti-Id which mimics the virus antigen would be important in virus diagnosis.

The question which we have addressed is whether one anti-idiotype generated by immunization with dengue virus glycoprotein epitopes is an internal image of the immunizing antigen bearing the dengue glycoprotein configuration. Such an antigen would be of value in diagnostic settings because of its non-hazardous character and its specificity for a specific dengue serotype. We have undertaken a project to produce an anti-Id, suitable for diagnostic work, from dengue type 2 monoclonal antibody in collaboration with the Howard Hughes hybridoma unit and with support in part by a grant from the Rockefeller Foundation.

MATERIALS AND METHODS AND RESULTS

Antibody purification and immunization of mice and rabbits.

The monoclonal antibody used in these studies was the 3H5 antibody which is a dengue 2 type specific antibody (Henchal et al., Am. J. Trop Med Hyg. 34(1),1985, pp 162-169.) This antibody was derived from fusion of P3x63Ag8 myeloma line with murine (BALB/C) spleen cells. This antibody has an IgG1 isotype and is specific for the E antigen of the structural virion. It's HI titer is reported by Henchal et al. to be 160, PRNT (50 PFU) 32,000, RIA 6.5 ($\log_{10}$) and infection enhancement titer <10.

Initially, purification of the antibody by protein A was difficult because IgG1 does not bind well to this matrix. Other methods tried were not satisfactory in regard to purity. However, by comparative experiments we determined that Protein A affinity column chromatography was acceptable with Affi-Gel Protein A using MAPS methodology and buffers produced by Bio-Rad (Richmond, Calif). With this buffer system, production of high titered purified antibody was achieved with high levels of recovery. Purity was confirmed by SDS-PAGE using silver staining of the gels. Antibody from either cell culture or from ascites

was recovered with excellent purity (See Figure 17 for illustration). Ascites were produced by inoculation of fused P3x63Ag8 cells into irradiated and anti-lymphocyte serum treated random bred Charles River mice. We found that yields were significantly increased as compared with immunization of mice bearing BALB/c markers. Mice and rabbits were given 2 injections of approximately 0.5 mg of antibody in the presence of Freund's adjuvant per inoculation at bi-weekly intervals.

Other rabbits were immunized with partially purified dengue type 2 virus grown in A. albopictus cells. Virus was concentrated by polyethene glycol (6000) precipitation and purified by sucrose gradient centrifugation. Approximately 100 micrograms of virus were inoculated intravenously into rabbits.

Preparation of affinity columns

Dengue 2 monoclonal antibody was purified and conjugated to cyanogen bromide activated Sepharose at 17 mg per gram of dried gel. In addition, Tete virus mouse polyclonal antibody was similarly conjugated to cyanogen bromide activated Sepharose.

Results with the murine system.

Spleen cells from two immunized mice were fused with SP2/0 myeloma cells. Over 200 spleen cell supernatant fluids were obtained from these fusion experiments. Each supernatant fluid was screened by ELISA for binding to anti-dengue 2 virus rabbit antibody (1:1000) or to a control rabbit antibody. The specificity of the anti-dengue 2 virus rabbit antibody was confirmed by antigen capture ELISA. When used as coating antibody at dilutions of 1:1000 or greater, the antibody captured dengue antigen as revealed by subsequent incubation with dengue antibody followed by addition of peroxidase-conjugated anti-mouse antibody. Cells producing supernatant fluids which bound only to the anti-dengue 2 virus rabbit antibody were cloned and re-examined by ELISA test. After recloning of nine different clones, we found that six of the subclones bound specifically to the anti-dengue 2 rabbit antibody. However, after growing the clones and freezing them, none of the six clones reacted specifically with the anti-dengue 2 rabbit antibody. All six clones possessed broadly reactive anti-rabbit activity. Further experiments were discontinued with these clones to pursue more promising work with rabbit anti-Id antibody.

Results with the rabbit system.

After one month, two rabbits were screened for anti-dengue 2 antibody reactivity by ELISA. Collected serum was run through the Tete affinity column followed by adsorption and elution from the anti-dengue 2 virus column. None of the immunized rabbits had antibody which bound to the anti-dengue monoclonal column. Both rabbit sera had considerable anti-mouse antibody activity which was removed by absorption to the anti-Tete affinity column.

Both rabbits were given a second injection of monoclonal antibody (0.50 mg) after one month. One of the two immunized rabbits died a week later of an illness thought to be bacterial in origin (Pseudomonas).

One of the immunized rabbits (rabbit one) had antibody which bound to the anti-dengue 2 column. This antibody was eluted and subjected to further testing after purification on a protein A column. (See Figure 18) Henceforth, this antibody is referred to as rabbit anti-Id.

Screening tests were conducted in two different ways. In the first instance, microtiter plates were coated with either dengue virus antibody or Sindbis virus antibody (1:100 dilutions). To these wells were added dilutions of rabbit antibody, followed by incubation, washing and addition of peroxidase conjugated anti-rabbit antibody. Secondly, rabbit antibody (putative anti-id) was used to coat plates at 1:100 dilutions, followed by addition of anti-dengue 2 or Sindbis virus antibody. By both tests, rabbit one possessed an anti-idiotypic antibody in the ELISA test.

The results of tests with rabbit anti-idiotypic antibody are given below. In these tests, the rabbit antibody was used as coating antibody at a 1:50 dilution. Murine antibodies, both polyclonal and monoclonal, were then incubated with the coating antibody followed by addition of peroxidase conjugated anti-mouse antibody (1:2000).

SERUM (MURINE)	SERUM DILUTION (RECIPROCAL)					
	50	200	800	3200	12800	25600
4G2(FLAVIVIRUS SPECIFIC MONOCLONAL	+	+	+	+	+	+
DENG 2 (POLYCLONAL)	+	+	+	+	-	-
RFV MONOCLONAL	-	-	-	-	-	-
DENG 2 MONOCLONAL	+	+	+	+	-	-
VEE POLYCLONAL	-	-	-	-	-	-
MVE POLYCLONAL	-	-	-	-	-	-
DENGUE 1 (POLYCLONAL)	-	-	-	-	-	-
DENGUE 3 (POLYCLONAL)	+	+	-	-	-	-
DENGUE 4 (POLYCLONAL)	-	-	-	-	-	-

Antibody from rabbit 1 blocked binding of dengue 2 antigen in a competition ELISA test. Microtiter plates were coated with rabbit anti-dengue 2 antibody. Dengue antigen was added, following which dengue 2 antibody, mixed with either PBS, rabbit #1 anti-Id antibody (1:50), or rabbit serum from an animal immunized with monoclonal antibody bearing an IgA isotype was added. The control rabbit antibody was provided by Dr. Kim Bottomly. The final step was addition of a mouse conjugate. The titer of the dengue 2 antibody was reduced 16 fold by incubation with the rabbit anti-Id antibody.

Results suggesting that serum from rabbit 1 contains an anti-idiotypic antibody bearing an internal image of the dengue 2 antigen are summarized as follows.

EVIDENCE THAT RABBIT 1 ANTIBODY IS AN ANTI-IDIOTYPE

CAPTURE OF DENGUE 2 ANTIBODY (ELISA)	COMPETITION FOR DENGUE 2 ANTIGEN (ELISA)	BINDING TO DENGUE 2 ANTIBODY (AFFINITY COLUMN)	GENERATION OF ANTI-[ANTI-ID]
+	+	+	**IN PROGRESS**

The results of these experiments, while encouraging, are currently inconclusive. Rabbits have been immunized with putative anti-Id from rabbit 1 in order to produce dengue neutralizing antibody specific for the E structural antigen of the virion. Additional tests are also required to determine if the antibody will capture polyclonal human antibody specific for dengue 2 virus. Our results suggest that the rabbit 1 serum may also react with dengue 3 antibody. We have no explanation for this result. Further bleedings from rabbit 1 will be subjected to testing for anti-Id activity.

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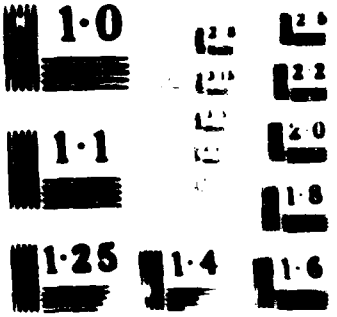
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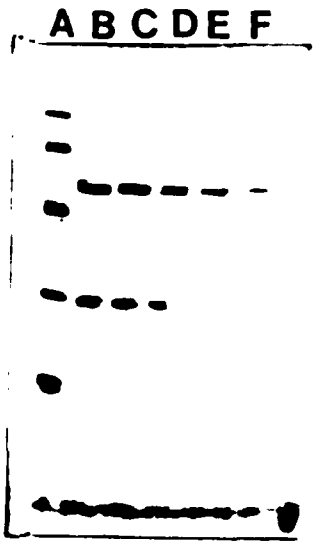
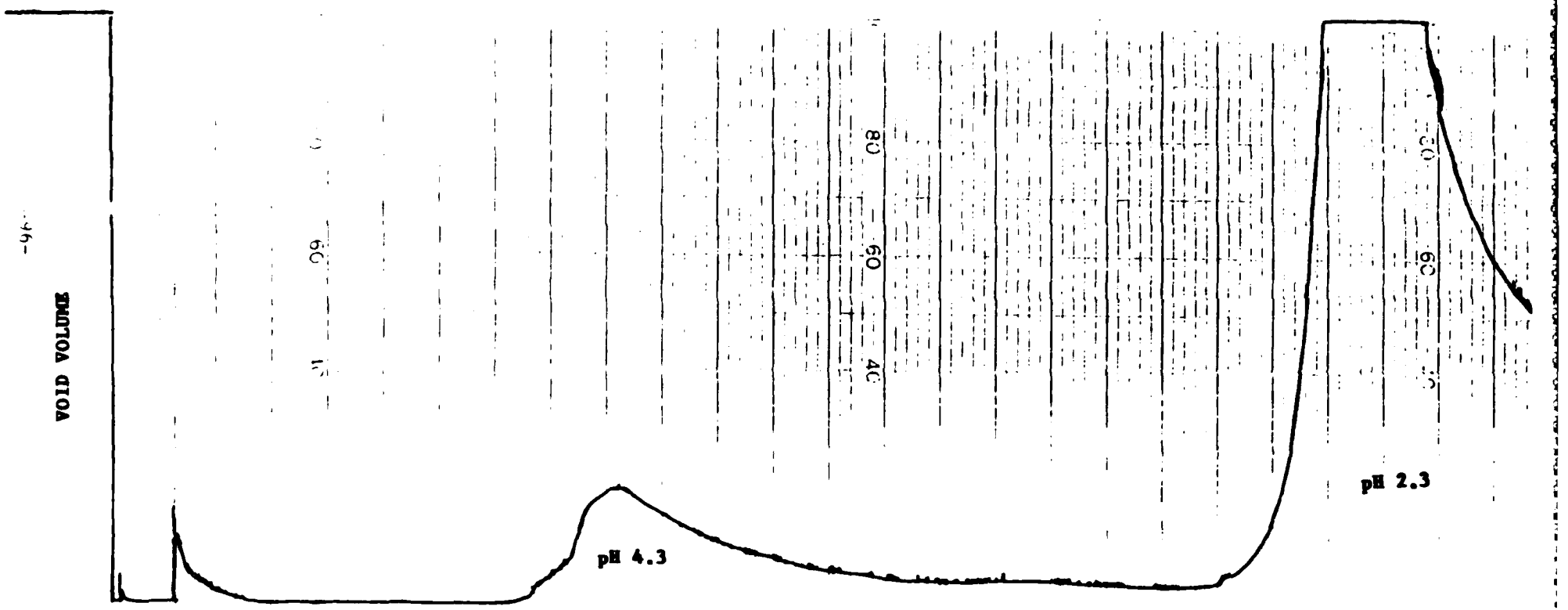


Figure 1

SDS-PAGE of Monoclonal Antibody 3H5 After Purification via Protein A-Affinity Chromatography. Lane A low molecular weight standards, Lanes B-F Monoclonal antibody in serial 2 fold dilutions: 20 ug, 10 ug, 5 ug, 2.5 ug, 1.25 ug of protein. Silver stain.

FIGURE 18

ELUTION PROFILE OF RABBIT ANTI-IDIOTYPE FROM A SEPHAROSE 4B COLUMN BEARING DENGUE 2 MONOCLONAL ANTIBODY



VI. COLLECTION OF LOW PASSAGE ARBOVIRUS REFERENCE STRAINS (R.B.Tesh).

In collaboration with the Subcommittee for the Collection of Low Passage Arbovirus Strains (SCLAS) of the American Committee on Arthropod-Borne Viruses (ACAV), an attempt has been made to establish a collection of low passage strains of selected arboviruses of public health importance. Previous reports about the collection were given in the 1981, 1982, 1983 and 1984 Annual Reports. During 1985, stocks of 40 more virus strains were prepared and lyophilized. These agents as well as their origin and passage history are listed in Table .

It is hoped that interested persons working in arbovirology will continue to submit samples of low passage virus strains from different geographical locations and time periods. We intend to create a data file with pertinent information on each virus in the collection. This information as well as the lyophilized virus stocks will be available to interested investigators at no cost. It is anticipated that this collection will prove to be an invaluable reference resource for future comparative studies on viral genetics, biochemistry, pathogenicity and antigenic relationships.

VII. DISTRIBUTION OF REAGENTS, WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH (A.J. Main, R.E. Shope, R.B. Tesh, D.L. Knudson and G.H. Tignor)

Distribution of reagents: A total of 1050 ampoules of virus stocks (307), antigens (497), and antisera (246) were distributed to 47 individuals in 20 countries plus 11 states and Puerto Rico. These reagents included 94 named and 106 unnamed viruses plus 135 polyvalent sera.

Table 37. Low Passage Strains - 1985

Virus	Strain	Passage	Source	Locality	Date
EEE	GML 903866	Vero #3	sentinel chicken	Bayano, Panama	1984
VSV-Ind	GML 903816	Vero #4	human(throat swab)	Panama City, Panama	1984
EEE	GML 900188	SM #2,Vero #1	horse brain	Gatuncillo, Panama	1962
Dengue-2	Ph.H 2172	AP-61 #2,C6/36 #1	human serum	Manila, Philippines	1983
JE	Ph.An 1242	Vero #1, C6/36 #1	pig blood	Santo Cristo, Philippines	1984
VEE(IAB)	G 8419	SM #2, Vero #2	horse	Sonora, Mexico	1972
VSV-Ind	L5-85	Vero #1	bovine teat	Guatemala	1984
VSV-NJ	L11-85	Vero #1	bovine teat	Panama	1985
VSV-NJ	L14-85	Vero #1	bovine teat	Costa Rica	1984
VSV-NJ	L35-85	Vero #1	bovine mouth	Nicaragua	1985
VSV-Ind	L30-84	Vero #1	bovine mouth	Costa Rica	1984
VSV-Ind	L125-84	Vero #1	bovine teat	Panama	1984
VSV-NJ	L264-84	Vero #1	bovine teat	Honduras	1984
VSV-NJ	L270-84	Vero #1	bovine mouth	Guatemala	1984
VSV-NJ	L243-84	Vero #1	bovine mouth	Belize	1984
EEE	lung #72	Vero #1	whooping crane	Patuxent Wildlife Research Center,MD	1984
EEE	FD #7829	Vero #2	Culiseta melanura	Pocomoke Cypress Swamp, MD	1983
EEE	FD #7830	Vero #2	Culiseta melanura	Pocomoke Cypress Swamp, MD	1983
EEE	Ma 2494	PCE #1, Vero #1	Culiseta melanura	Raynham, MA	Sept.1977
EEE	Ma 2020	PCE #1, Vero #1	Culiseta melanura	Halifax, MA	Aug. 1978

Table 37 (Continued) Low Passage Strains - 1985

Virus	Strain	Passage	Source	Locality	Date
EEE	Ma 1058	PCE #1, Vero #1	Culiseta melanura	Halifax, MA	July 1979
EEE	Ma 1833	PCE #1, Vero #1	Culiseta melanura	Halifax, MA	Aug. 1980
EEE	Ma 396	PCE #1, Vero #1	Culiseta melanura	New Bedford, MA	Aug. 1982
EEE	Ma 848	PCE #1, Vero #1	Culiseta melanura	Easton, MA	Sept.1983
EEE	Ma 1313	PCE #1, Vero #1	Culiseta melanura	Raynham, MA	Sept.1984
Chikungunya	RSU1	Vero #2	human serum	Ambon Island, Indonesia	1985
YF	INS-347613	C6/36 #3	human serum	Antioquia, Colombia	1985
DEN-1	INS-347869	C6/36 #3	human serum	Caqueta, Colombia	1985
EEE	VO80784/1	Vero #1	horse brain	Florida	1984
EEE	VO82085/2	Vero #1	horse brain	Florida	1985
VSV-NJ	L8-82	Vero #1	bovine epithleium	Honduras	1982
VSV-NJ	L14-82	Vero #1	" "	Costa Rica	1982
VSV-NJ	L67-82	Vero #1	" "	Guatemala	1982
VSV-Ind	L2-83	?,Vero #1	" "	Honduras	1983
VSV-NJ	L153-83	Vero #1	" "	Nicaragua	1983
VSV-Ind	L51-85	Vero #1	" "	Costa Rica	1985
VSV-Ind	L111-85	Vero #1	" "	Salvador	1985
VSV-NJ	L183-83	Vero #1	" "	Panama	1983
VSV-NJ	L177-85	Vero #1	" "	Honduras	1985
Ross River	Aus.Ar.96614	SM#1,C6/36#1	Aedes sp.	New South Wales,Australia	1983

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